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**SEPTEMBER 1951**
**VOLUME 88 NUMBER 3**

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
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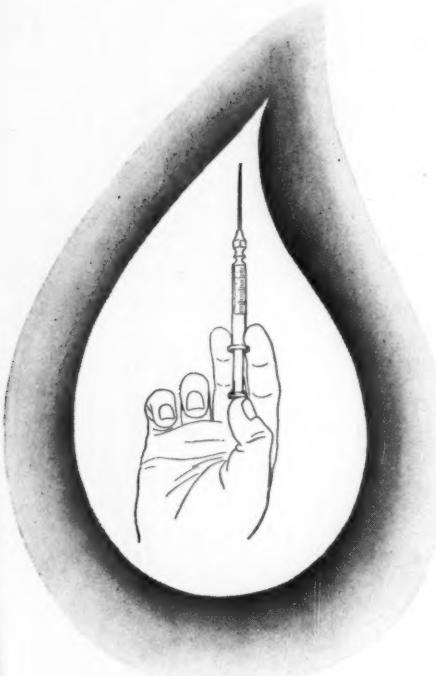

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**BIBLIOGRAPHY**

(1) Tompsett, R., and McDermott, W., Am. J. Med. *7*: 371-381, Sept. 1949. (2) Tompsett, R., Ann. Otol., Rhin. & Laryng. *57*: 181, March 1948. (3) Sweany, H. C., Dis. Chest *15*: 631-656, June 1949. (4) Lincoln, S., Science News Letter *55*: 307, May 14, 1949. (5) Semans, J. H., J. M. A. Georgia *38*: 477-480, Nov. 1949. (6) Domon, C. M., Kilbourne, P. C., and King, E. Q., Amer. Rev. Tuberc. *60*: 564, 575, Nov. 1949. (7) Nagley, M. M., Brit. M. J. *1*: 248, Jan. 28, 1950 (in correspondence). (8) Committee on Medical Research and Therapy, American Trudeau Society, Am. Rev. Tuberc. *61*: 436-440, March 1950. (9) Carr, D. T., Hinshaw, H. C., Pfuetze, K. H., and Brown, H. A., Dis. Chest *16*: 801-821, Dec. 1949. (10) Odell, J. M., Dis. Chest *16*: 818, Dec. 1949. (11) (Editorial) New England J. Med. *240*: 736, May 5, 1949. (12) Keefer, C. S., Ann. Int. Med. *33*: 582-589, Sept. 1950. (13) Marsh, D. F., W. Va. Med. J. *45*: 280-284, Oct. 1949. (14) Johnson, H. M., J. Invest. Dermat. *15*: 61-66, July 1950. (15) Hinshaw, H. C., Personal communication. (16) Lincoln, N. S., Horton, R., Stokes, A. M., Monroe, J., and Riggins, H. M., Am. Rev. Tuberc. *62*: 572-581, Dec. 1950. (17) Carr, D. T., Brown, H. A., Hogson, C. H., and Heilman, F. R., J. A. M. A. *143*: 1223-1225, Aug. 5, 1950. (18) Jacoby, A., Goldberg, W., Sobel, N., and Rosenthal, T., Am. J. Syph., Gonor. & Ven. Dis. *34*: 185-186, March 1950.


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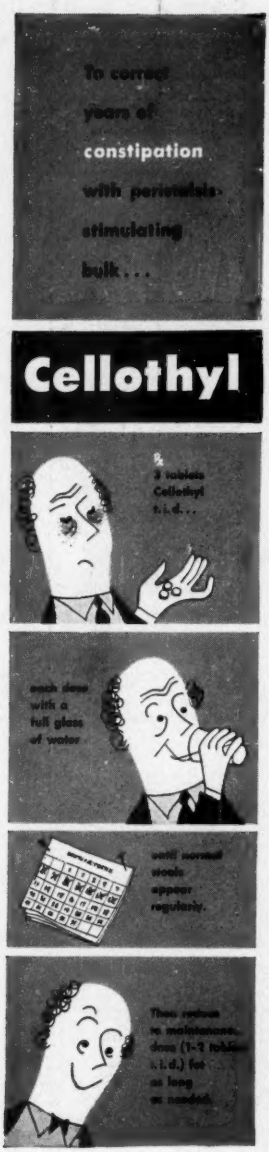
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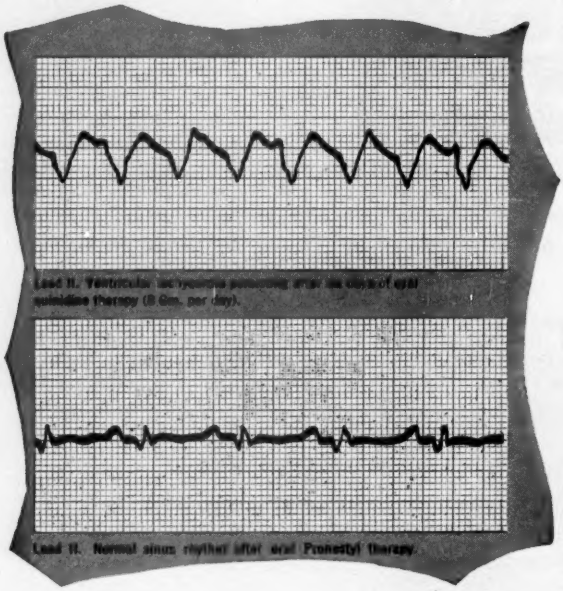

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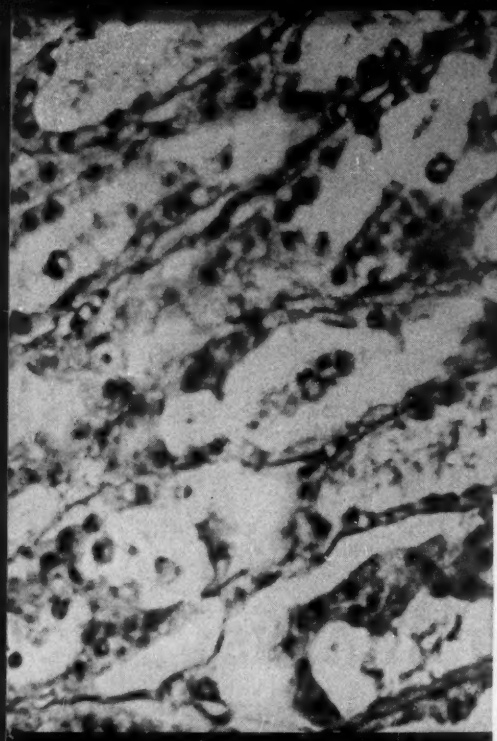

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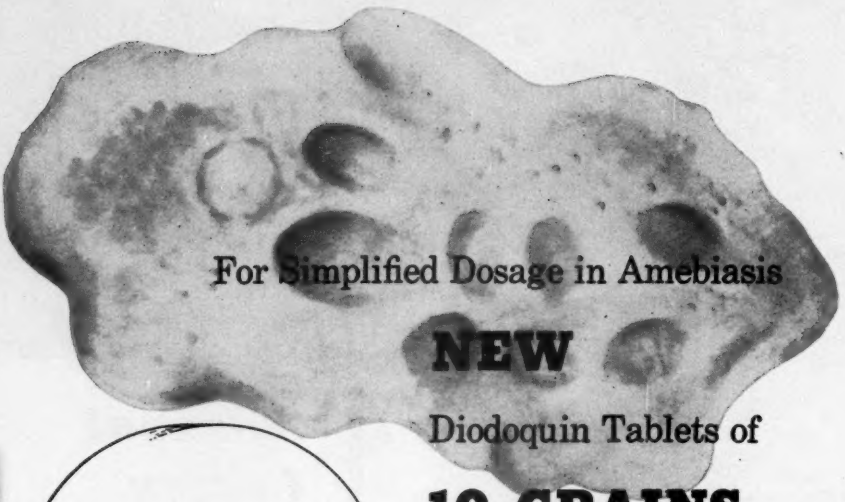

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THE A. M. A. ARCHIVES OF INTERNAL MEDICINE is published by the American Medical Association for the advancement of scientific medicine in the United States and for the reporting of research and observation in the domain of internal medicine.

Manuscripts for publication, books for review and correspondence relating to the editorial management should be sent to the Chief Editor, Dr. Paul S. Rhoads, 535 North Dearborn Street, Chicago 10. Communications regarding subscriptions, reprints, etc., should be addressed, A. M. A. ARCHIVES OF INTERNAL MEDICINE, American Medical Association, 535 North Dearborn Street, Chicago 10.

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A. M. A.
ARCHIVES OF INTERNAL MEDICINE

VOLUME 88 SEPTEMBER 1951 NUMBER 3

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# EFFECT OF LARGE DOSES OF AUREOMYCIN ON HUMAN LIVER

MARK H. LEPPER, M.D.
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CHARLES K. WOLFE, M.D.
HYMAN J. ZIMMERMAN, M.D.
ESTON R. CALDWELL Jr., M.D.
AND
HAROLD W. SPIES, M.D.
WASHINGTON, D. C.
AND
HARRY F. DOWLING, M.D.
CHICAGO

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From the Department of Preventive Medicine, University of Illinois College of Medicine, Chicago; the George Washington and Georgetown Medical Divisions, Gallinger Municipal Hospital, and the Departments of Medicine, George Washington and Georgetown Universities, Washington, D. C.

1. (*a*) Brainerd, H.; Lennette, E. H.; Meiklejohn, G.; Bruyn, H. B., Jr., and Clark, W. H.: Symposium on Antibiotics: Clinical Evaluation of Aureomycin, J. Clin. Invest. **28**:992-1005 (Sept., Pt. 1) 1949. (*b*) Dowling, H. F.; Lepper, M. H.; Caldwell, E. R., Jr.; Whelton, R. L., and Sweet, L. K.: Aureomycin in Various Infections: Report of 180 Cases and Review of Clinical Literature, M. Ann. District of Columbia **18**:335-344 (July) 1949. (*c*) Finland, M.; Collins, H. S., and Paine, T. F., Jr.: Aureomycin, New Antibotic: Results of Laboratory Studies and of Clinical Use in 100 Cases of Bacterial Infections, J. A. M. A. **138**:946-949 (Nov. 27) 1948. (*d*) Finland, M.; Collins, H. S.; Gocke, T. M., and Wells, E. B.: Present Status of Aureomycin Therapy, Ann. Int. Med. **31**:39-52 (July) 1949. (*e*) Harvey, J. C.; Mirick, G. C., and Schaub, I. G.: Symposium on Antibiotics: Clinical Experience with Aureomycin, J. Clin. Invest. **28**:987-991 (Sept., Pt. 1) 1949. (*f*) Sanders, M.; Rumdall, J. M.; Solomon, C.; Soret, M. G., and Ricci, N. I.: Symposium on Antibiotics: Further Experiences with Intravenous Aureomycin Therapy; Study of 116 Cases, ibid. **28**:1006-1013 (Sept., Pt. 1) 1949. (*g*) Spink, W. W., and Yow, E. M.: Aureomycin: Present Status in Treatment of Human Infections, J. A. M. A. **141**:964-966 (Dec. 3) 1949. (*h*) Woodward, T. E.: Chloromycetin and Aureomycin: Therapeutic Results, Ann. Int. Med. **31**:52-82 (July) 1949.

2. Herrell, W. E., and Heilman, F. R.: Aureomycin: Studies on Absorption, Diffusion and Excretion, Proc. Staff Meet., Mayo Clin. **24**:157-166 (March 30) 1949. Dowling, H. F.; Lepper, M. H., and Caldwell, E. R.: Unpublished data. Randall, R. V.; Taylor, R. W., and Wellman, W. E.: Intravenous Administration of Aureomycin Including Studies on the Concentration in the Serum, ibid. **24**:605-611 (Dec. 7) 1949.

panied intramuscular or subcutaneous administration. An occasional allergic-type reaction has also been reported.[3]

We have administered aureomycin [4] intravenously to a number of seriously ill patients. Because of the severity of their illnesses, they were given large intravenous doses of aureomycin, occasionally for a long period and, in many cases, oral doses of aureomycin in addition. The great majority of the patients who received aureomycin intravenously showed no evidence of toxic or side reactions other than those mentioned above. A few patients, however, who were given what we now consider to be excessive doses of aureomycin intravenously, showed clinical and/or laboratory evidence of injury to the liver. We are presenting in this paper clinical data on these patients and, where available, the results of the pathologic examinations.

## RESULTS

During the past 30 mo. we have treated 61 adults and 42 children with aureomycin intravenously (Table 1). Aureomycin was administered orally concurrently to 14 of these patients. In none of the 89 patients who received aureomycin intravenously alone, in doses of 1.5 to 9.0 mg. per kilogram of body weight every six hours, was any evidence of liver dysfunction developed or exhibited. Specific studies of liver function, however, were performed for only a few of these patients. Twenty-eight of the 89 patients received treatment for more than 48 hr., but only six were treated intravenously for more than three days.

Among the 14 patients who received aureomycin by both the oral and the intravenous routes, seven displayed signs of liver dysfunction. Enlargement of the liver developed in each of them. Four had clinical jaundice, and hyperbilirubinemia was present in an additional patient. Five of these patients died, and a biopsy was performed on a sixth. In these six patients there were definite pathologic changes present in the liver, which will be described in the individual case reports that follow.

## REPORT OF CASES

PATIENT 1.—E. T., a 10-yr.-old girl, weighing 20 kg., was admitted on Aug. 5, 1949, to Gallinger Municipal Hospital, Washington, D. C., with a diagnosis of paralytic poliomyelitis. The weakness of the right arm and leg promptly disappeared, but on Aug. 17 signs of meningitis developed. Repeated cultures of the spinal fluid revealed Pseudomonas aeruginosa. She was given 3 gm. of streptomycin daily for two days and then 1 gm. a day for seven days. At the same time, a 2-gm. dose of sulfadiazine was given, followed by 0.5 gm. every four hours for nine days. With this therapy all signs and symptoms of meningitis disappeared. Within two days after the treatment was discontinued, however, the signs of meningitis reappeared, and Pseudomonas was again cultured from the spinal fluid. On Aug. 31 the patient was given two intravenous doses of 300 mg. of aureomycin hydrochloride (with sodium glycinate buffer) six hours apart. In addition, aureomycin was given orally in doses of 250 mg. every three hours. Because the patient's condition was not improving, the oral dose of 2 gm. a day was supplemented with 500 mg. of aureomycin hydrochloride (buffered with sodium glycinate) given intravenously at eight-hour intervals. By Sept. 6 she had become listless and had begun to vomit intermittently. The next day, icterus appeared and the liver was found to be enlarged and tender. Vomiting became severe and persistent. Treatment with gantrisin® (3,4-dimethyl-5-sulfanilamido-isoxazole) was begun in doses of 2 gm. every eight hours. Laboratory tests on Sept. 7

3. Peck, S. M., and Feldman, F. F.: Sensitivity Reactions to Aureomycin, J. A. M. A. **142**:1137-1139 (April 15) 1950.

4. Aureomycin used in this study was supplied by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

revealed no increase in the mild anemia which had been present on admission. The direct serum-bilirubin level was 19 mg. per 100 cc. Signs of meningitis and progressive hepatic disease continued. On Sept. 9, bile was present in the urine, the cephalin-flocculation reaction was 4+, and the thymol turbidity, 5.1 units. The prothrombin time was 51% of normal. On Sept. 10 the patient vomited 1 pint (473 cc.) of blood and started passing tarry stools. The total serum bilirubin was

TABLE 1.—*Data on 103 Patients Treated with Aureomycin Intravenously*

| Intravenous Aureomycin Therapy | | | | Oral Aureomycin Therapy | | | | |
|---|---|---|---|---|---|---|---|---|
| Dose, Mg./Kg. | Interval Between Doses, Hr. | No. of Injections | Total Intravenous Dose, Gm. | Dose, Mg./Kg. | Interval Between Doses, Hr. | Total Oral Dose, Gm. | No. of Patients Treated | No. of Patients with Evidence of Liver Damage |
| 1.5 | 6 | 2 | 0.2 | ... | .. | .... | 1 | 0 |
| 3.0 | 6 | 1 | 0.2 | ... | .. | .... | 2 | 0 |
| 4.5 | 6 | 1 | 0.3 | ... | .. | .... | 6 | 0 |
| 5.0* | 6 | 6 | 0.15 | ... | .. | .... | 1 | 0 |
| 5.0* | 6 | 7 | 0.35 | ... | .. | .... | 1 | 0 |
| 5.0* | 6 | 8 | 0.2† 1.6 | ... | .. | .... | 4 | 0 |
| 5.0 | 6 | 10 | 2.5 | ... | .. | .... | 1 | 0 |
| 5.0* | 6 | 11 | 0.55† 1.0 | ... | .. | .... | 2 | 0 |
| 5.0* | 6 | 24 | 0.96 | ... | .. | .... | 1 | 0 |
| 6.0 | 6 | 5 | 2.0 | ... | .. | .... | 1 | 0 |
| 6.0 | 6 | 6 | 2.4 | ... | .. | .... | 1 | 0 |
| 8.0 | 6 | 1 | 0.5 | ... | .. | .... | 11 | 0 |
| 8.0 | 6 | 2 | 1.0 | ... | .. | .... | 3 | 0 |
| 8.0 | 6 | 3 | 1.5 | ... | .. | .... | 3 | 0 |
| 8.0 | 6 | 4 | 2.0 | ... | .. | .... | 4 | 0 |
| 8.0 | 6 | 6 | 3.0 | ... | .. | .... | 5 | 0 |
| 8.0 | 6 | 12 | 6.0 | ... | .. | .... | 2 | 0 |
| 8.0 | 6 | 40 | 20.0 | ... | .. | .... | 2 | 0 |
| 9.0* | 6 | 2 | 0.2 | ... | .. | .... | 1 | 0 |
| 9.0 | 6 | 3 | 1.5 | ... | .. | .... | 1 | 0 |
| 9.0* | 6 | 6 | 0.3† 0.9 | ... | .. | .... | 3 | 0 |
| 9.0 | 6 | 6 | 1.5 | ... | .. | .... | 1 | 0 |
| 9.0* | 6 | 7 | 0.5† 1.3 | ... | .. | .... | 4 | 0 |
| 9.0* | 6 | 8 | 0.7 | ... | .. | .... | 6 | 0 |
| 9.0 | 6 | 8 | 5.0 | ... | .. | .... | 2 | 0 |
| 9.0* | 6 | 9 | 0.5† 1.8 | ... | .. | .... | 5 | 0 |
| 9.0* | 6 | 10 | 0.5† 3.6 | ... | .. | .... | 6 | 0 |
| 9.0 | 6 | 10 | 6.0 | ... | .. | .... | 2 | 0 |
| 9.0* | 6 | 11 | 0.7† 3.0 | ... | .. | .... | 4 | 0 |
| 9.0* | 6 | 12 | 1.1 | ... | .. | .... | 1 | 0 |
| 9.0* | 6 | 14 | 1.9 | ... | .. | .... | 1 | 0 |
| 9.0* | 6 | 17 | 3.1 | ... | .. | .... | 1 | 0 |
| 8.0 | 24 | 6‡ | 2.4 | 250 | 3 | 13.6 | 1 | 1§ |
| 8.0 | 6 | 5 | 2.5 | 250 | 3 | 24.5 | 1 | 0 |
| 8.0 | 6 | 10 | 5.0 | 250 | 3 | 20.0 | 1 | 0 |
| 8.0 | 6 | 12 | 6.0 | 250 | 3 | 9.0 | 1 | 0 |
| 8.0 | 6 | 12 | 6.0 | 250 | 3 | 14.0 | 1 | 1§ |
| 8.0 | 6 | 50‖ | 25.6 | 250 | 3 | 32.0 | 1 | 0 |
| 8.0 | 6 | 1 | 0.5 | 500 | 3 | 12.0 | 1 | 0 |
| 8.0 | 6 | 6 | 3.0 | 500 | 3 | 24.0 | 1 | 0 |
| 8.0 | 6 | 12 | 6.0 | 500 | 3 | 36.0 | 1 | 1¶ |
| 8.0 | 6 | 7 | 3.5 | 750 | 4 | 22.5 | 1 | 1§ |
| 10.0 | 6 | 12 | 7.4 | 500 | 3 | 10.5 | 1 | 1§ |
| 12.0* | 6 | 21 | 12.5# | 250 | 3 | 22.0 | 1 | 1§ |
| 12.0 | 6 | 16 | 12.0 | 750 | 3 | 24.0 | 1 | 0 |
| 16.0 | 6 | 24 | 24.0 | 250 | 3 | 10.0 | 1 | 1¶ |
| | | | | | | Total | 103 | 7 |

* Child.
† Initial dose.
‡ Also received one intravenous injection of 400 mg.
‖ Also received two 1-gm. doses intravenously.
§ Died.
¶ Recovered.
# Also received two intravenous doses of 300 mg.
The weight of the 61 adult patients was estimated in most instances. All the children were weighed.

19.5 mg. per 100 cc., of which 7.5 mg. was direct-reacting bilirubin. At that time, aureomycin therapy was discontinued and chloramphenicol therapy was started. During the administration of chloramphenicol in doses of 1.5 gm. a day, the patient's condition became progressively worse and the liver continued to enlarge. Paralytic ileus and pulmonary edema developed on Sept. 14, and the area of cardiac dulness was increased. The patient died two days later.

Ps. aeruginosa was repeatedly cultured from specimens of spinal fluid throughout the course of the disease. On Sept. 12 and 14, the cephalin-flocculation reaction was 4+, and the thymol turbidity, 4 and 7 units, respectively. On Sept. 13 the total serum bilirubin was 22.5 mg. per 100 cc.

At necropsy, the liver was enlarged and yellow and appeared greasy. The capsule was smooth. The cut section was also yellow and greasy. Microscopically, there were extensive changes of the liver cells, most marked in the central portion of the lobules and consisting of vacuolation and fragmentation of the cytoplasm. The cells closest to the central vein showed autolysis, with complete loss of cytoplasmic structure (Figs. 1 and 2). There was also congestion of the sinusoids.

PATIENT 2.—F. W., a 28-yr.-old woman, was admitted to Gallinger Municipal Hospital on June 9, 1949, and was found to have healed rheumatic valvulitis, staphylococcic bacterial endocarditis, and congestive heart failure. Because the patient's condition did not improve

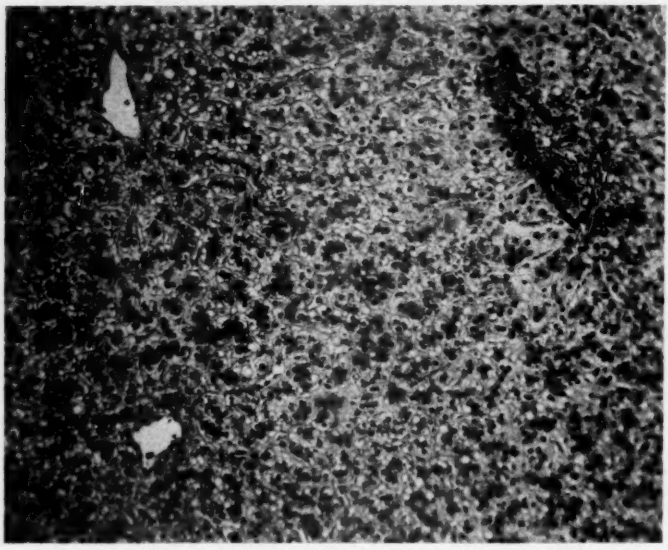

Fig. 1.—Necropsy section of liver of Patient 1 (E. T.); × 100.

after eight days of therapy with 1,000,000 units of penicillin given intramuscularly every two hours, the treatment was changed on June 25 to administration of 250 mg. of aureomycin orally every three hours. Because of a tooth extraction on July 7, she received an additional 400 mg. of aureomycin (in a leucine buffer) intravenously. A change in therapy was made Aug. 1 because petechiae, fever, and positive blood cultures had returned and in-vitro studies indicated a synergistic action of aureomycin and penicillin. Accordingly, 1,000,000 units of penicillin was given intramuscularly every two hours, combined with 2 gm. of aureomycin a day given by mouth. When apparent drug fever developed, the penicillin treatment was discontinued on Aug. 9.

Because the patient was vomiting and not taking all the medicament orally, she received daily intravenous injections of 500 mg. of aureomycin (in an *l*-leucine buffer) from Aug. 6 through 11. Her condition seemed to improve except for the congestive heart failure, which was believed to be the cause of the persistent hepatomegaly and ascites. Oral aureomycin therapy was continued until Sept. 1. A total of 136 gm. of aureomycin had been given during

a period of 68 days. When a relapse occurred on Sept. 14, 4 gm. of chloramphenicol was given each day for 34 days. Signs of heart failure and rheumatic activity continued, and the patient left the hospital on her own responsibility on Oct. 17. Since the hepatomegaly was attributed to congestive failure, no studies of liver function were done. The patient was observed at home, where she died on Nov. 2, 1949.

At necropsy, the liver was large, the capsule was smooth, and the yellow, greasy appearance of both the capsular and the cut surfaces was striking. Microscopically, cytoplasmic vacuolation and fragmentation were observed, confined to the central half of the lobules (Fig. 3).

PATIENT 3.—L. L., a 69-yr.-old man, was admitted to Gallinger Municipal Hospital, Sept. 8, 1949, with an obstructing duodenal ulcer. Because his symptoms could not be controlled by medical therapy, a subtotal gastrectomy was done on Oct. 30. At that time, intramuscular injections of 100,000 units of penicillin were given at three-hour intervals and were continued

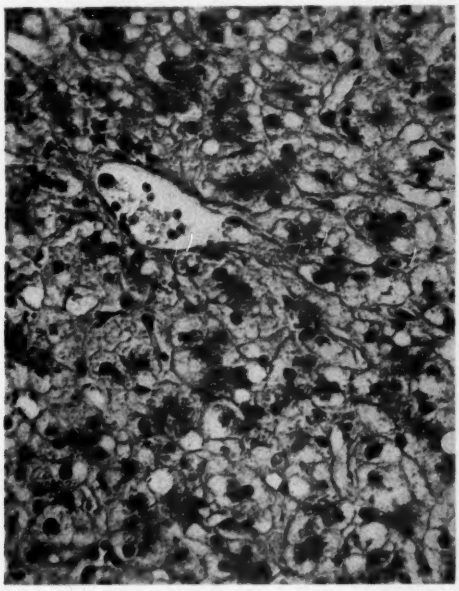

Fig. 2.—Liver from Patient 1 (E. T.); showing marked vacuolation and fragmentation of the cytoplasm. Hematoxylin and eosin stain; × 300.

until he died. Consolidation of the lower lobe of the right lung was detected, and, starting on Nov. 14 intravenous injections of 500 mg. of aureomycin hydrochloride (buffered with sodium glycinate) were given every six hours for four days. In addition, 2 gm. of aureomycin was given daily by stomach tube from Nov. 14 to 21. On Nov. 21 a jejunostomy was performed, and at operation the liver was noted to be enlarged. This was correlated in retrospect with an isolated observation of an icterus index of 19 units on Nov. 7 (a finding which had previously been attributed to transfusion hemolysis). On Nov. 22 clinical icterus was first observed. On Nov. 21 streptomycin therapy was begun and was continued until the time of the patient's death on Nov. 25.

At necropsy, the liver was yellow and greasy and microscopically showed cytoplasmic fragmentation and vacuolation of hepatic cells similar to that described in the two previous cases but, as in the second case, largely confined to the central half of the lobule (Fig. 4).

PATIENT 4.—D. R., a 20-yr.-old woman, was admitted to Gallinger Municipal Hospital on Nov. 23, 1949, with typhoid of three days' duration. After the diagnosis was established on Nov. 26, the patient was given aureomycin hydrochloride (buffered with sodium glycinate), 1 gm. every six hours intravenously, and aureomycin hydrochloride, 250 mg. every three hours by mouth. Within three days, the elevated temperature had receded. On Nov. 30, however, although defervescence had occurred, the patient became icteric, and enlargement and tenderness of the liver were observed. Aureomycin therapy was discontinued at this time. The signs of hepatitis progressed during the next five days and then began to recede. On Dec. 1, 3, and 6, the results of the two-hour urobilinogen tests were 0.19, 0.21, and 0.92 Ehrlich unit, respectively. On Dec. 1, 5, and 8, the total serum bilirubin values were 6.6 (direct, 2.8), 7.4 (direct, 3.5), and 4.4 (direct, 1.8) mg. per 100 cc., respectively; the thymol turbidity was 2.4, 4.6, and 2.4 units, respectively, and the cephalin-cholesterol-flocculation readings were consistenly 2+. There was 3.8 units of alkaline phosphatase on Dec. 8.

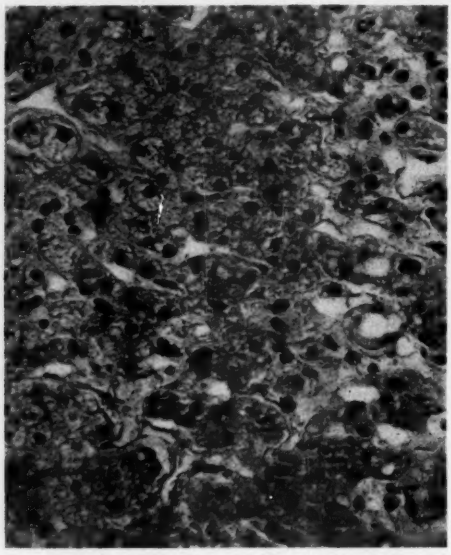

Fig. 3.—Liver from Patient 2 (F. W.), showing vacuolation of the cytoplasm. Hematoxylin and eosin stain; × 300.

A liver biopsy was performed on Dec. 10, 10 days after aureomycin therapy had been discontinued. Numerous large and small vacuoles, presumably fat, were seen in the parenchymal cells throughout the section (Fig. 5). These changes were similar to but of lesser degree than those described in the previous cases. The distribution of the changes could not be evaluated in this specimen obtained by biopsy.

On Dec. 27, when the patient suffered a relapse of the typhoid, oral chloramphenicol therapy was instituted in doses of 3 gm. initially and 250 mg. every three hours by mouth. There was no clinical evidence of a recurrence of liver damage, and the patient recovered without further difficulty.

PATIENT 5.—F. R., a 38-yr.-old man with a history of chronic alcoholism, was admitted to Gallinger Municipal Hospital on March 2, 1950, with meningitis caused by Pneumococcus type IV. Treatment was promptly started with 1,000,000 units of penicillin given every two hours intramuscularly, 750 mg. of aureomycin given orally every four hours, and 500 mg. of

aureomycin (buffered with sodium glycinate) given intravenously at six-hour intervals. Although clinical improvement had begun within 36 hr. of admission, the urine output decreased progressively. The specific gravity was low, and albuminuria and hematuria were present. As the blood urea nitrogen increased, the patient went into coma with convulsions and died on the fifth hospital day. The total intravenous dose of aureomycin during a two-day period was 3.5 gm. In addition, 22.5 gm. was administered orally in five days.

At necropsy, the liver showed the same yellow, greasy appearance grossly and the microscopic cytoplasmic changes decribed in the previous cases. In addition, a moderate increase in fibrous tissue in the portal spaces, as well as intralobularly, and some increase in bile canaliculi were present. The microscopic diagnosis was early portal cirrhosis, upon which the described cytoplasmic changes seemed superimposed.

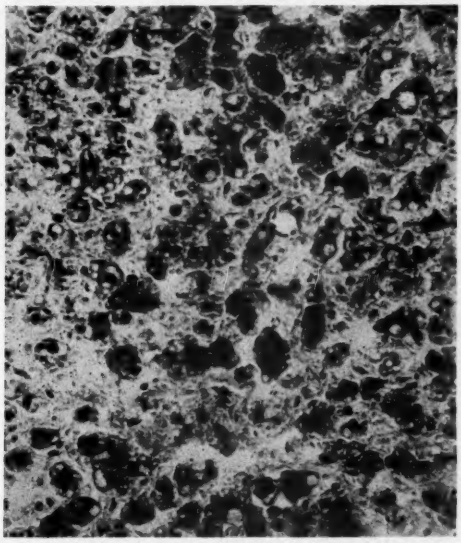

Fig. 4.—Liver from Patient 3 (L. L.), showing cytoplasmic vacuoles and necrosis. Hematoxylin and eosin stain; × 300.

PATIENT 6.—I. W., a 36-yr.-old Negro man, was admitted to Gallinger Municipal Hospital on Oct. 26, 1949, with signs and symptoms of bacterial endocarditis. Hemolytic Staphylococcus pyogenes var. aureus was obtained from each of three separate blood cultures taken after admission. Oral aureomycin administration was started in doses of 500 mg. every three hours. Since the patient's condition did not respond clinically, intravenous therapy with aureomycin with sodium glycinate, in doses of 500 mg. every six hours, was added on Nov. 6. On Nov. 7 the serum bilirubin was 0.6 mg. per 100 cc. On Nov. 9, the third day of the high intravenous doses of aureomycin, the patient became icteric. Since a hemolytic Staphylococcus was obtained from a blood culture and since there was no clinical improvement, the intravenous aureomycin therapy was discontinued, and on Nov. 13 streptomycin was given in doses of 1 gm. every six hours, along with 1 gm. of aureomycin orally every three hours. Twelve hours later, the temperature was normal and the jaundice was considerably less. Two days later, the temperature rose to 100 F. and remained elevated for the next 10 days. By this time, the jaundice had disappeared and it was necessary to reduce the oral dose of aureomycin to 500 mg. every three hours because of nausea and vomiting.

After 21 days of streptomycin and aureomycin therapy, the patient was still febrile, the leucocyte count was 11,200 per cubic millimeter, and the pulse rate ranged from 90 to 110 a minute. Streptomycin therapy was discontinued on Dec. 2. Aureomycin therapy, in doses of 500 mg. intravenously every six hours and 500 mg. orally every three hours, was tried, but after three days the liver and spleen became palpable. On Dec. 6, the sulfobromophthalein (bromsulphalein®) sodium test showed 15% retention of the dye, the thymol turbidity was 4 units, and the cephalin-flocculation reaction, 1+. On Dec. 7, administration of 2,000,000 units of crystalline penicillin every two hours was started and all aureomycin therapy was discontinued. The following day the patient was digitalized. After three days of administration of high doses of penicillin, the temperature remained over 100 F. and the pulse rate was 130 a minute. The patient by this time had again become jaundiced, although the enlargement of the liver was receding.

Since in-vitro studies had revealed a synergistic effect with combinations of aureomycin and penicillin, oral administration of aureomycin, 750 mg. every three hours, was added to the

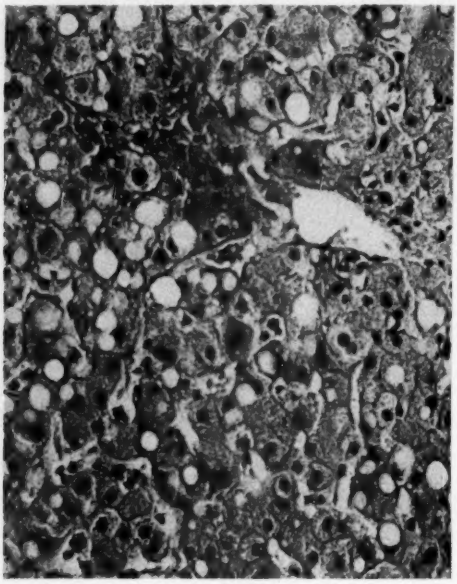

Fig. 5.—Liver from Patient 4 (D. R.), showing fatty metamorphosis (needle-biopsy specimen). Hematoxylin and eosin stain; × 300.

penicillin therapy. During this period the jaundice was clearing, although on Dec. 20 the serum bilirubin was still 4.5 mg. per 100 cc. The patient was febrile, with the temperature between 100 and 101 F., for two weeks; the pulse rate remained elevated, 100 to 120 a minute, and the leucocyte counts were 18,000 to 19,000 per cubic millimeter during this period. For the next three weeks, the temperature was below 99.8 F. rectally; the patient's condition appeared to improve clinically, and he made an uneventful recovery.

PATIENT 7.—B. F., a 50-yr.-old white woman, was admitted to the Municipal Contagious Disease Hospital, Chicago, with a history of symptoms suggestive of meningitis of two days' duration. Physical examination revealed a comatose, obese, critically ill patient with signs of meningeal irritation. The diagnosis of meningitis was confirmed by the results of lumbar puncture. The spinal fluid was purulent and contained an alpha hemolytic Streptococcus which failed to type with the antipneumococcic serums available. Because of her obesity, the patient was given 1.2 gm. of aureomycin hydrochloride with sodium glycinate intravenously every six

hours for two doses, and then 500 mg. every six hours for 10 doses. In addition, 500 mg. of aureomycin was given every three hours by stomach tube. Penicillin was administered intravenously in doses of 1,000,000 units every two hours. The patient's condition became progressively worse, and she died on the third hospital day.

At autopsy (pathologic report by Dr. Paul Szanto, hospital pathologist) the liver was observed to be tremendously enlarged with fatty metamorphosis. The fat globules were seen in most of the cells of the central and middle zones. The globules were considerably larger than the fat vacuoles observed in all the other patients described. There was moderate periportal fibrosis, but the architecture was well preserved. The fatty metamorphosis seen in this patient, in contradistinction to that observed in the other patients in this group, was similar to that seen in many other clinical conditions.

For the purpose of determining whether laboratory evidences of liver dysfunction could be observed in subjects who were administered a combination of oral and intravenous therapy with aureomycin, the following two patients were given 500 mg. of aureomycin hydrochloride (buffered with sodium glycinate) intravenously at six-hour intervals for three days, in addition to oral therapy. Liver-function studies were performed before and after treatment, as follows: sulfobromophthalein excretion,[5] thymol turbidity,[6] and cephalin-cholesterol flocculation.[7] Data on these patients are not included in Table 1.

TABLE 2.—*Results of Liver-Function Tests in Two Patients Before and After Intravenous Aureomycin Therapy*

| Patient | Days from Beginning of Intravenous Therapy | Sulfobromophthalein Retention, % | Thymol Turbidity, Units | Cephalin Flocculation |
|---|---|---|---|---|
| R. H. (before intravenous aureomycin therapy) | .. | 2 | 4.6 | 3+ |
| | 2 | 8 | 5.1 | 2+ |
| | 4 | 8 | 6.2 | 3+ |
| | 9 | 5 | 5.6 | 4+ |
| | 21 | 8 | 6.2 | 4+ |
| E. L. (before intravenous aureomycin therapy) | .. | 7 | 1.4 | 1+ |
| | | | 2.4 | 1+ |
| | 4 | 23 | 3.0 | 2+ |
| | 9 | 15 | 4.3 | 2+ |

PATIENT 8.—R. H. had chronic bronchiectasis and was convalescing from an attack of pneumonia. Aureomycin had been administered orally in doses of 250 mg. every three hours and later every six hours, beginning Dec. 27, 1949. On Jan. 9, 1950, intravenous aureomycin therapy was added and continued for three days. Before the intravenous aureomycin therapy was started, the thymol turbidity was 4.6 units and the cephalin-flocculation reaction, 3 +; there was 2% retention of sulfobromophthalein sodium (Table 2). On the second, fourth, ninth, and 21st day after therapy was started, the thymol turbidity was 5.1, 6.2, 5.6, and 6.2 units; the cephalin flocculation, 2+, 3+, 4+, and 4+ and sulfobromophthalein sodium retention, 8, 8, 5, and 8% respectively.

PATIENT 9.—E. L. was recovering from acute pyelonephritis. She had received aureomycin orally in doses of 250 mg. every three hours for 17 days, and this therapy was continued along with the intravenous administration. Before aureomycin was given intravenously thymol-turbidity values on two different days were 1.4 and 2.4 units and cephalin-flocculation reactions were 1+. There was also 7% retention of sulfobromophthalein sodium before treatment. Four

5. Mateer, J. G.; Baltz, J. I.; Marion, D. F., and MacMillan, J. M.: Liver Function Tests, J. A. M. A. **121**:723-728 (March 6) 1943.

6. Maclagan, N. F.: Thymol Turbidity Test: New Indicator of Liver Dysfunction, Nature, London **154**:670-671 (Nov. 25) 1944.

7. Hanger, F. M.: Serological Differentiation of Obstructive from Hepatogenous Jaundice by Flocculation of Cephalin-Cholesterol Emulsions, J. Clin. Invest. **18**:261-269 (May) 1939.

and nine days after the start of therapy, the thymol turbidity was 3.0 and 4.3 units, the cephalin flocculation, 2+ and 2+, and the sulfobromophthalein retention, 23 and 15%, respectively (Table 2).

During the past 30 months, we have administered aureomycin intravenously to 96 other patients. None of these patients exhibited clinical evidence of hepatic damage which could be attributed to the drug. Table 1 presents detailed information on these patients. The majority of them received 8 to 9 mg. per kilogram of body weight intravenously at six-hour intervals with or without supplemental oral administration of aureomycin. The duration of therapy was one to three days in most patients. Only an occasional patient was treated four or more days. The largest dose given to any patient who did not display evidence of liver disease was 12 mg. per kilogram of body weight intravenously every six hours for four days, plus the same amount every three hours orally for the same four days and an additional 12 weeks.

## COMMENT

The clinical experiences outlined above suggested to us that aureomycin might, when given in large doses by the combined intravenous and oral routes, produce damage to the liver. Because of this, a series of experiments has been done on mice, which is reported on elsewhere in this issue.[8] This work has shown that aureomycin and terramycin can produce in the livers of mice histologic changes which simulate those seen in each of the patients reported on here, except Patient 7. In the latter patient the microscopic observations differed in that the fat was in large intracellular globules and the architecture of the cells not involved was quite normal. In the mouse liver and in sections from the other patients there was much small vacuolation of the cytoplasm, with irregular fragmentation. For this reason, we feel that the pathologic changes in Patient 7 did not necessarily represent the same process as those in the other patients and those produced in the mice, but the details are given since this patient had manifest liver disease.

The amount of aureomycin or terramycin required to cause damage to the liver, however, is considerably more than that which is commonly used or needed for therapeutic purposes; this is borne out by the fact that the combined experiments of other investigators and ourselves [1] have brought to light little evidence of injury to the liver which could be attributed to the use of aureomycin or terramycin. In addition to the liver damage in the patients who are reported on in the present paper, each of whom received what we now believe to be excessive doses of aureomycin, Yesner and Kunkel [9] and Gewin and Friou [10] have reported that fatty infiltration may occur in the liver after prolonged oral administration of aureomycin. Among approximately 1,300 patients to whom we have administered aureomycin only by oral route,[11] we have not observed clinical evidence of damage to the liver which could be attributed to the drug.

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8. Lepper, M. H.; Zimmerman, H. J.; Carroll, G.; Caldwell, E. R., Jr.; Spies, H. W.; Wolfe, C. K., and Dowling, H. F.: Effect of Large Doses of Aureomycin, Terramycin, and Chloramphenicol on Livers of Mice and Dogs, A. M. A. Arch. Int. Med., this issue, p. 284.

9. Yesner, R., and Kunkel, P.: Preliminary Observations on Effect of Aureomycin, Terramycin, Tibione, Combined Tibione and Streptomycin, and Chloromycetin on Morphology of Liver in Man, Yale J. Biol. & Med. **23**:299-304 (Feb.) 1951.

10. Gewin, H. M., and Friou, G. J.: Manifestations of Vitamin Deficiency During Aureomycin and Chloramphenicol Therapy of Endocarditis Due to Staphylococcus Aureus: Report of Case, Yale J. Biol. & Med. **23**:332-338 (Feb.) 1951.

11. Dowling, H. F., and Lepper, M. H.: Unpublished data.

Results of liver-function tests on human subjects receiving aureomycin have revealed no significant alterations,[1a, f] even after a dose as large as 1 gm. given as a single intravenous injection.[1f] Harned and his associates [12] performed sulfobromophthalein-retention tests in dogs before and after administration of 0.122 to 0.316 gm. of aureomycin per kilogram of body weight intravenously for eight days. None of the animals showed a significant increase in dye retention. Bryer and his co-workers [13] reported that there was no change in the histologic appearance of mouse livers after a total dose of 400 mg. per kilogram of body weight given subcutaneously over a nine-day period (44 mg. per kilogram per day).

Among 103 patients to whom we gave aureomycin intravenously, the seven reported on in detail had liver damage which appeared to be related to the use of the antibiotic. Each of these patients had, for one reason or another, a dose that might be considered excessive. Thus, in patient 1 (E. T.), the intravenous dose of 500 mg. every six hours in a 20-kg. child (100 mg. per kilogram per 24 hr.), in addition to oral doses of aureomycin, was given because of the relative resistance of the organism causing her meningitis. In the second patient (F. W.) the oral dose of 500 mg. every three hours (60 mg. per kilogram per 24 hr.) was supplemented by daily intravenous doses of 500 mg. These undoubtedly produced high peak concentrations. Since in this patient the pathologic changes were discovered at autopsy, a considerable time after aureomycin treatment was discontinued, and since chloramphenicol was administered in the interim, the relation of the aureomycin to the damage is not certain. The third patient (L. L.) received 500 mg. intravenously four times a day (50 mg. per kilogram per day) in addition to 2 gm. a day by mouth. In Patient 4 (D. R.) with typhoid, a large intravenous dose of 1 gm. every six hours (60 mg. per kilogram per day), plus an oral dose of 250 mg. every three hours (30 mg. per kilogram per day), was given. In Patient 5 (F. R.) with pneumococcic meningitis the effect of the large dose given, 500 mg. intravenously every six hours (33 mg. per kilogram per 24 hr.) and 750 mg. orally every four hours, may have been enhanced by the oliguria which occurred apparently as a result of a lower-nephron nephrosis.

Patient 6 (I. W.) was given 500 mg. of aureomycin orally every three hours for nine days and 500 mg. intravenously every six hours for three days before enlargement of the liver appeared. This subsided when intravenous aureomycin therapy was discontinued, even though the oral dose was increased to 1 gm. every three hours. After 108 gm. of aureomycin had been given orally in the intervening 19 days, therapy with a combination of 500 mg. of aureomycin orally every three hours and 500 mg. intravenously every six hours was given accompanied with hepatomegaly and jaundice. This likewise receded when intravenous aureomycin therapy was discontinued, even though aureomycin was administered by the oral route for the next eight weeks.

Patient 7 (B. F.) received two very large doses of 1.2 gm. each at six-hour intervals because of her very large size. This dose was followed by concurrently administered intravenous and oral therapy.

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12. Harned, B. K.; Cunningham, R. W.; Clark, M. C.; Cosgrove, R.; Hine, C. H.; McCauley, W. J.; Stokey, E.; Vessey, R. E.; Yuda, N. N., and Subbarow, Y.: Pharmacology of Duomycin, Ann. New York Acad. Sc. **51**:182-210 (Nov. 30) 1948.

13. Bryer, M. S.; Schoenbach, E. B.; Bliss, E. A., and Chandler, C. A.: Treatment of Experimental Infections with Aureomycin, Ann. New York Acad. Sc. **51**:254-266 (Nov. 30) 1948.

That the dose of 500 mg. intravenously every six hours in an adult, along with 1 to 2 gm. a day by mouth, can alter liver function has been shown in the two patients who received this dose after preliminary liver-funtion studies were done, and in whom there was no other apparent cause for the diminution of liver function which was observed. On the other hand, that the liver is not clinically damaged regularly by intravenous use of aureomycin is indicated by the fact that we have observed no such damage in 96 other patients who received the drug (Table 1). Although many of these patients received doses for a shorter time than the patients reported on, nevertheless several patients received doses comparable with those which produced liver damage. It would appear that 9 mg. per kilogram of body weight given intravenously four times a day is close to the toxic threshold, at least for some human livers.

It must be emphasized that the six patients from whom we obtained liver tissue for examination had evidence of potential or demonstrable liver damage prior to any treatment. Patient 2 (F. W.) had severe heart failure with hepatomegaly. Patient 5 (F. R.) was a known chronic alcoholic. Patient 3 (L. L.) had an icterus index of 19 during his first postoperative week before receiving any aureomycin. The remaining three patients had severe febrile infections which are known to be capable of producing liver damage. The extent of previous liver damage in our patients is difficult to determine, but we believe that these patients were abnormally susceptible to any of the hepatotoxic agents. The role of pre-existing liver damage in potentiating the hepatotoxic effect of an agent is exemplified by the well-known fact that the toxicity to the liver of agents such as carbon tetrachloride is greatly enhanced in the chronic alcoholic patient or in the malnourished animal.

As a matter of fact, patients with infectious hepatitis have been treated with aureomycin orally [14] without any evidence that the severity of the disease was increased. Likewise, several patients with liver damage caused by infectious mononucleosis [15] treated with aureomycin orally showed rapid improvement, both clinically and as measured by laboratory tests. Furthermore, aureomycin has been reported to have a beneficial effect in the treatment of hepatic coma [16] and of chronic liver disease.[17]

It is possible that aureomycin has the effect of increasing the metabolism of the liver, so that in the usual amounts it is beneficial, but in excessive doses, harmful. Excessive amounts administered therapeutically, for instance, may act in a manner analogous to that of excessive amounts of dextrose given by the intravenous route.[18]

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14. Shaffer, J. M.; Farquhar, J. D.; Stokes, J., Jr., and Sborov, V. M.: Studies on Use of Aureomycin in Hepatic Disease: I. Aureomycin Therapy in Acute Viral Hepatitis, Am. J. M. Sc. **220**:1-5 (July) 1950.

15. Gruskin, B. J.: Aureomycin in Acute Infectious Mononucleosis, Ann. Int. Med. **31**:678-681 (Oct.) 1949.

16. Farquhar, J. D.; Stokes, J., Jr.; Whitlock, C. M., Jr.; Bluemle, L. W., Jr., and Gambescia, J. M.: Studies on Use of Aureomycin in Hepatic Disease: III. Note on Aureomycin Therapy in Hepatic Coma, Am. J. M. Sc. **220**:166-172 (Aug.) 1950.

17. Shaffer, J. M.; Bluemle, L. W., Jr.; Sborov, V. M., and Neefe, J. R.: Studies on Use of Aureomycin in Hepatic Disease: IV. Aureomycin Therapy in Chronic Liver Disease, with Note on Dermal Sensitivity, Am. J. M. Sc. **220**:173-182 (Aug.) 1950.

18. Bollman, J. L.: Liver Glycogen and the Protection of Liver Cells, in Conference on Liver Injury, 1949: Transactions of Conference, New York, Josiah Macy, Jr., Foundation, 1950, p. 115.

From the clinical data presented, the liver damage would seem to be reversible if administration of the drug is stopped soon enough, as was done in Cases 4 and 6 (D. R. and I. W.). The presence of pathologic changes in the livers of Patients 1 and 2 (E. T. and F. W.) for six and 60 days, respectively, after aureomycin therapy was discontinued suggests that the damage may persist for some time, although it must be noted that each of these patients received a considerable amount of chloramphenicol, which may have added to the damage.

Our observations would suggest that there is an upper limit for the dose of aureomycin which can be administered intravenously. If this limit is exceeded, injury to the liver may occur. Until further data can be accumulated on aureomycin, it would seem wise to limit the intravenous dose to 40 mg. per kilogram of body weight for each 24-hr. period, divided into four doses, if intravenous therapy is used alone. If aureomycin is given orally at the same time, or if liver damage is already present, smaller intravenous doses should be employed. Larger doses may be used when the possible risk is outweighed by the gravity of the illness, and, even here, the duration of treatment should be shortened as much as possible.

There is no evidence that oral administration of aureomycin in the dosage range commonly employed will cause any clinical evidence of injury to the liver.

## SUMMARY AND CONCLUSION

One hundred three patients were given large doses of aureomycin in treatment of serious infections; 14 of them received both oral and intravenous aureomycin therapy. Among these 14 patients, seven showed clinical evidences of liver dysfunction. Necropsy examinations on five and a biopsy specimen from one of these patients showed pathologic changes in the liver cells.

Two subjects were given aureomycin intravenously and were studied by means of serial liver-function tests. These showed diminution of liver function. The pathologic changes in the liver appeared to be reversible when the condition was recognized early and aureomycin therapy was promptly discontinued.

Intravenous doses of aureomycin totalling less than 2 gm. a day in adults were not accompanied with evidence of liver dysfunction or injury, nor have these conditions been observed in more than 1,300 patients to whom we have administered aureomycin orally.

The doses of aureomycin which caused these changes in the liver are now considered to be excessive. It is recommended, until further evidence is accumulated, that aureomycin should not be given intravenously in large doses. It is suggested that when aureomycin is administered orally in addition no more than 1 gm. of aureomycin a day should be given by the intravenous route. When adjuvant oral therapy is not employed, 2 gm. of aureomycin intravenously a day would seem to be the maximum dose. For children a maximum intravenous dose of 40 mg. per kilogram of body weight is recommended.

# EFFECT OF LARGE DOSES OF AUREOMYCIN, TERRAMYCIN, AND CHLORAMPHENICOL ON LIVERS OF MICE AND DOGS

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DURING the course of clinical trials to determine the value of intravenous aureomycin therapy in various infections, we encountered several patients who exhibited jaundice, which, in some cases, was accompanied with pathologic changes in the liver. Our suspicions were aroused that the hepatic changes might be related to the administration of aureomycin because they did not appear to be the result of the disease for which the patient was being treated. Furthermore, all the patients who displayed jaundice had received very large doses of aureomycin. The details of these cases are reported in the preceding article in this issue.[1] As a result of those observations, we decided to investigate the possible toxic effects of aureomycin on the livers of mice and dogs.

Several reports in the literature suggested that mice tolerate aureomycin well. Up to 200 mg. per kilogram of body weight per day given orally for eight weeks caused no evident damage, even when the mice received 100 mg. per kilogram per day for four weeks preceding use of the higher dose. Mice also tolerated single doses of 200 mg. per kilogram of body weight given intraperitoneally and 1,500 mg. per kilogram given orally, as shown by Harned and his co-workers.[2] The same investigators, however, reported that when aureomycin was administered by the intravenous route the medial lethal dose ($L.D._{50}$) of aureomycin was 134 mg. per

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1. Lepper, M. H.; Wolfe, C. K.; Zimmerman, H. J.; Caldwell, E. R., Jr.; Spies, H. W., and Dowling, H. F.: Effect of Large Doses of Aureomycin on Human Liver, A. M. A. Arch. Int. Med., this issue, p. 271.

2. Harned, B. K.; Cunningham, R. W.; Clark, M. C.; Cosgrove, R.; Hine, C. H.; McCauley, W. J.; Stoken, E.; Vessey, R. E.; Yuda, N. N., and Subbarow, Y.: Pharmacology of Duomycin, Ann. New York Acad. Sc. **51**:182-210 (Nov. 30) 1948.

kilogram of body weight. Bryer and associates [3] found that 400 mg. given in divided doses over a nine-day period caused no histologic changes in mouse livers. György and his associates [4] stated that aureomycin added to the diet in doses of 0.1 gm. a day gave some protection to the livers of rats which had been placed on a diet known to cause necrosis of the liver.

In this paper we present evidence that aureomycin is capable of producing gross and microscopic changes, possibly pathologic, in mouse livers when administered parenterally in moderately large doses at intervals over a period of several days, the changes becoming apparent within 24 hr. after the first dose.

Because of the similarity in the antimicrobial action of aureomycin. terramycin,[5] and chloramphenicol,[6] the toxicity of the latter two compounds was also tested. P'an and associates [7] have shown that the administration of terramycin to dogs is accompanied with the development of abnormal results on liver-function tests. Our data show that changes in the liver also occur in mice after terramycin administration. The results obtained with chloramphenicol administration are not so conclusive as those obtained with use of the other two compounds.

TABLE 1.—*Experiment 1: Effect of Intraperitoneally Administered Aureomycin on Livers of Mice*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| A | Saline control group: 0.2 cc. 0.85% NaCl intraperitoneally daily for 7 days; killed on 8th day | 48 | 44 | 4 | .. | .. | .. |
| B | Aureomycin group: 2 mg. aureomycin HCl and 1 mg. sodium glycinate in 0.2 cc. 5% dextrose solution intraperitoneally daily for 7 days; killed on 8th day | 47 | 2 | 3 | 12 | 16 | 14 |

## PROCEDURE

Hybrid mice, weighing 20 to 25 gm. each, were used throughout the experiment. They were fed purina® fox chow ("checkers") without supplement.

In the first experiment (Table 1) one group of 47 mice was given 2 mg. (80 to 100 mg. per kilogram of body weight) of aureomycin hydrochloride,[8] buffered with 1 mg. of sodium glycinate dissolved in 0.2 cc. of 5% dextrose solution, intraperitoneally daily for seven days. A control group of 48 mice received a daily intraperitoneal injection of 0.2 cc. of 0.85% (isotonic) sodium chloride solution for the same period.

In the second experiment (Table 2) six groups of mice were used. The animals in Group 1 received 2 mg. (80 to 100 mg. per kilogram of body weight) of aureomycin hydrochloride intraperitoneally for seven days. Those in Group 2 received identical injections of aureomycin

3. Bryer, M. S.; Schoenbach, E. B.; Bliss, E. A., and Chandler, C. A.: Treatment of Experimental Infections with Aureomycin, Ann. New York Acad. Sc. **51**:254-266 (Nov. 30) 1948.

4. György, P.; Stokes, J., Jr.; Smith, W. H., and Goldblatt, H.: Studies on the Use of Aureomycin in Hepatic Disease: II. Effect of Aureomycin on Experimental Dietary Hepatic Necrosis, Am. J. M. Sc. **220**:6-11 (July) 1950.

5. Terramycin used in this study was supplied by Chas. Pfizer & Company, Inc., Brooklyn 6.

6. Chloramphenicol used in this study was supplied by Parke, Davis & Company, Detroit 32.

7. Cited by Hobby, G. L.: Personal communication to the authors.

8. Aureomycin used in this study was supplied by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

hydrochloride, diluted similarly, but buffered with 1 mg. of sodium glycinate. In Group 3 injections were given of the same materials as in the case of Group 2, but the subcutaneous route of administration was employed. The mice in one of the control groups (Group 4) were given 1 mg. of sodium glycinate in 0.2 cc. of 0.85% (isotonic) sodium chloride solution intraperitoneally each day; those in the second control group (Group 5), 0.2 cc. of 0.001 M hydrochloric acid intraperitoneally once a day, and those in Group 6, 0.2 cc. of 0.85% (isotonic) sodium chloride solution each day by the same route. On the eighth day the animals in both experiments were killed by breaking the neck, and the livers were removed immediately.

TABLE 2.—*Experiment 2: Effect of Route of Administration of Aureomycin, pH of Solution, and Presence of Sodium Glycinate on Changes in Mouse Liver*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| 1 | 2 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl intraperitoneally daily; killed on 8th day | 8 | .. | .. | 1 | 4 | 3 |
| 2 | 2 mg. aureomycin HCl plus 1 mg. sodium glycinate in 0.2 cc. 0.85% NaCl intraperitoneally daily; killed on 8th day | 8 | .. | .. | 1 | 4 | 3 |
| 3 | 2 mg. aureomycin HCl with 1 mg. sodium glycinate in 0.2 cc. 0.85% NaCl subcutaneously daily; killed on 8th day | 8 | .. | .. | .. | 4 | 4 |
| 4 | 1 mg. sodium glycinate in 0.2 cc. 0.85% NaCl intraperitoneally daily; killed on 8th day | 12 | 7 | 3 | 2 | .. | .. |
| 5 | 0.2 cc. 0.001 M hydrochloric acid intraperitoneally daily; killed on 8th day | 10 | 2 | 3 | 5 | .. | .. |
| 6 | 0.2 cc. isotonic NaCl solution intraperitoneally daily; killed on 8th day | 8 | 5 | 3 | .. | .. | .. |

TABLE 3.—*Experiment 3: Effect of Intraperitoneally Administered Aureomycin on Livers of Mice in Comparison with Livers of Mice on a Similar Food Intake Without Aureomycin*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| Control | 0.2 cc. 5% dextrose solution intraperitoneally daily until killed (fed amount of food that mice receiving aureomycin ate); killed as follows: | | | | | | |
| | 3d day | 3 | 1 | 1 | 1 | .. | .. |
| | 5th day | 5 | .. | 1 | 3 | .. | 1 |
| | 7th day | 6 | 2 | 3 | .. | .. | 1 |
| | 9th day (8 doses NaCl solution) | 8 | 2 | 4 | 2 | .. | .. |
| | Total | 22 | 5 | 9 | 6 | .. | 2 |
| Aureomycin | 2 mg. aureomycin HCl with 1 mg. sodium glycinate in 0.2 cc. 5% dextrose solution intraperitoneally daily; food ad lib.; killed as follows: | | | | | | |
| | 24 hr. | 6 | .. | .. | 2 | 4 | .. |
| | 3d day | 3 | .. | .. | .. | 3 | .. |
| | 5th day | 4 | .. | .. | .. | 4 | .. |
| | 7th day | 5 | .. | .. | .. | .. | 5 |
| | 9th day | 5 | .. | .. | .. | .. | 5 |
| | Total | 23 | .. | .. | 2 | 11 | 10 |

In the third experiment (Table 3), since it was felt that the food intake of the mice given aureomycin may have been lessened because of peritoneal or subcutaneous irritation and inflammation, the food intake of the control animals was limited to that which the animals that were receiving aureomycin had eaten during the preceding 12 hr. Both groups of mice received injections as in the first experiment, except that 0.2 cc. of a 5% dextrose solution was given to the control animals instead of a similar volume of isotonic sodium chloride solution. The mice in the control group received no food for the first 12 hr. At the end of that time

the amount consumed by the aureomycin-treated animals in the preceding 12 hr. was given to the control mice. Every 24 hr. throughout the remainder of the experiment the control mice were fed an amount similar to that eaten by those in the aureomycin group during the preceding 24 hr. In this manner the control animals were given somewhat less to eat than the experimental lot. Some of the mice receiving aureomycin were killed 24 hr. after the first injection, and animals from both groups were killed on the third, fifth, seventh, and ninth days after the initial injection. Because of the fact that two of the control animals showed 4+ changes similar to those observed in the treated animals, further studies were needed. In experiment 3 the animals were housed in groups of eight. Consequently the exact food intake of each animal was not known; hence, it is possible that some animals were ingesting more than others which were actually starving.

TABLE 4.—*Experiment 4: Effect of Subcutaneous Administration of Aureomycin on Livers of Mice in Comparison with Livers of Mice on Similar Food Intake*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| Control | 0.2 cc. of 0.85% NaCl subcutaneously for 7 days (fed ad lib.); killed on 8th day | 10 | 3 | 6 | 1 | 0 | 0 |
| Paired control | 0.2 cc. of 0.85% NaCl subcutaneously for 7 days (fed amount that mice receiving aureomycin ate); killed on 8th day | 10 | 3 | 7 | 0 | 0 | 0 |
| Aureomycin | 2 mg. aureomycin HCl with 1 mg. sodium glycinate in 0.2 cc. of 0.85% NaCl subcutaneously daily (fed ad lib.); killed on 8th day | 10 | 0 | 0 | 3 | 4 | 3 |

TABLE 5.—*Experiment 5: Effect of Parenterally and Orally Administered Aureomycin, Terramycin, and Chloramphenicol on Livers of Mice*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| 1 | 2 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl orally daily; killed on 8th day | 8 | 4 | 1 | 3 | .. | .. |
| 2 | 2 mg. terramycin HCl in 0.2 cc. 0.85% NaCl orally daily; killed on 8th day | 9 | 4 | 3 | 2 | .. | .. |
| 3 | 2 mg. chloramphenicol in 0.2 cc. 0.85% NaCl orally daily; killed on 8th day | 10 | 10 | .. | .. | .. | .. |
| 4 | 0.2 cc. 0.85% NaCl intraperitoneally and orally daily; killed on 8th day | 9 | 6 | 2 | 1 | .. | .. |
| 5 | 2 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl intraperitoneally daily; killed on 8th day | 9 | .. | .. | .. | 2 | 7 |
| 6 | 2 mg. terramycin HCl in 0.2 cc. 0.85% NaCl intraperitoneally daily; killed on 8th day | 10 | .. | .. | .. | 4 | 6 |
| 7 | 2 mg. chloramphenicol in 8% alcohol-water solution intraperitoneally daily; killed on 8th day | 4 | 2 | 2 | .. | .. | .. |
| 8 | Same as Group 7; died spontaneously on 7th day | 3 | 1 | 2 | .. | .. | .. |

Experiment 4 (Table 4) was done to control the effect of food intake more accurately. Each mouse was housed individually, and the mice were "paired fed" in such a way that the intake of each control animal was limited to that ingested by its treated counterpart during the preceding 12 hr. The animals in one group received 2 mg. of aureomycin hydrochloride with sodium glycinate, 1 mg., in 0.2 cc. of 0.85% sodium chloride solution subcutaneously daily for seven days, and those in the other group the same dose of sodium chloride solution. In addition, a third group of animals was treated, each of which received 0.2 cc. of isotonic sodium chloride solution subcutaneously daily. The mice in this group, however, were allowed to eat ad libitum, and the intake was measured. All the animals were killed on the eighth day.

In the fifth experiment the procedures outlined in Table 5 were used. The drugs marked "orally" were given by intubation. The control animals (Group 4) received both an intraperitoneal injection and a dose of sodium chloride solution by intubation. Some difficulty was encountered in producing a stable solution of chloramphenicol. Accordingly, the exact dose used is not known in all cases, since some precipitation occurred during injection.

The sixth experiment contained the groups listed in Table 6. (No significant changes were made in the method.) The heat-inactivated aureomycin was prepared by incubating the material at 37 C. in a water bath for 18 hr. By this means all but 1/500 or 1/1000 of the antibacterial activity was lost. Again, some difficulty was experienced in obtaining an accurate dose of chloramphenicol.

TABLE 6.—*Experiment 6: Effect of Large Intraperitoneal Doses of Chloramphenicol and Terramycin, Large Oral Doses of Aureomycin, and Subcutaneous Doses of Heat-Inactivated Aureomycin on Mouse Livers*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| 1 | 8 mg. terramycin HCl in 0.2 cc. 0.85% NaCl intraperitoneally; died after 1 day (1 dose) | 7 | 5 | 1 (postmortem autolysis) | 1 | .. | .. |
| 2 | 8 mg. terramycin HCl in 0.2 cc. 0.85% NaCl intraperitoneally daily; died after 2 days (2 doses) | 1 | .. | 1 | .. | .. | .. |
| 3 | 8 mg. terramycin HCl in 0.2 cc. 0.85% NaCl intraperitoneally daily; killed after 5 days | 2 | .. | 1 | .. | 1 | .. |
| 4 | 8 mg. chloramphenicol in 3% alcohol-water solution intraperitoneally daily; killed after 5 days | 5 | 1 | 4 | .. | .. | .. |
| 5 | 8 mg. chloramphenicol in 3% alcohol-water solution intraperitoneally daily; found dead after 2 days | 3 | 1 | 2 | .. | .. | .. |
| 6 | 10 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl orally daily; died on 3d day | 1 | .. | .. | 1 | .. | .. |
| 7 | 10 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl orally daily; found dead after 1 day | 2 | 1 | .. | 1 | .. | .. |
| 8 | 10 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl orally daily; killed after 5 days | 8 | 2 | 3 | 2 | 1 | .. |
| 9 | 2 mg. aureomycin HCl in 0.2 cc. 0.85% NaCl subcutaneously daily; killed after 5 days | 10 | .. | 5 | 3 | 2 | .. |
| 10 | 0.2 mg. heat-inactivated aureomycin HCl in 0.2 cc. 0.85% NaCl subcutaneously daily; killed after 5 days | 10 | 2 | 4 | 3 | 1 | .. |
| 11 | 0.1 cc. 0.85% NaCl intraperitoneally daily; 3 found dead; 4 killed after 5 days | 5 | 4 | .. | .. | 1 | .. |
| 12 | 0.1 cc. 0.85% NaCl intraperitoneally daily; found dead on 2d day | 2 | 2 | .. | .. | .. | .. |
| 13 | 0.4 cc. 3% alcohol-water solution intraperitoneally daily; killed after 5 days | 8 | 6 | 2 | .. | .. | .. |

TABLE 7.—*Effect of Intravenously Administered Aureomycin on Livers of Mice in Comparison with Livers of Mice Fed Equal Amounts of Food*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| Control | 0.2 cc. of 0.85% NaCl intravenously daily for 6 days (food limited to that eaten by aureomycin group); killed on 7th day | 10 | 6 | 2 | 1 | 1 | 0 |
| Experimental | 2 mg. aureomycin HCl with 1 mg. sodium glycinate intravenously in 0.2 cc. of 0.85% NaCl intravenously daily for 6 days (fed ad lib.); killed on 7th day | 10 | 0 | 2 | 0 | 4 | 4 |

In an attempt to reduce the possible effect of tissue irritation from the injection of aureomycin, a seventh study (Table 7) was done in which the aureomycin group received 2 mg. of the antibiotic with 1 mg. of sodium glycinate diluted in 0.2 cc. of 0.85% sodium chloride solution intravenously daily for six days, while the control group received a similar dose of sodium chloride solution. In this instance the animals were "paired fed" as in Experiment 4. The animals were killed on the seventh day.

The tails of the animals receiving aureomycin, however, became quite inflamed and necrotic. Consequently, an eighth experiment (Table 8) was performed. In this study, 4 mg. of aureomycin hydrochloride with 2 mg. of sodium glycinate was dissolved in 0.4 cc. of isotonic sodium

chloride solution and given intravenously as a single injection. The control group received a comparable dose of sodium chloride solution intravenously. The animals were killed 18 hr. later. In addition, five completely untouched animals were killed for examination.

Because of the success obtained with the single-intravenous-injection technique, this was felt to be the best method for investigating the relation of dose to effect and to compare the action of aureomycin, terramycin, and chloramphenicol. Therefore, several dose schedules of each of the three antibiotics were administered as single intravenous doses, and a control group of animals was also included (Table 9). All animals were killed 18 hr. after the drug admin-

TABLE 8.—*Effect on Livers of Mice of Single Large Dose of Aureomycin Administered Intravenously*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ |
| Control | No procedure | 5 | 4 | 0 | 1 | 0 | 0 |
| Control | 0.4 cc. of 0.85% NaCl intravenously; killed in 18 hr. | 10 | 9 | 0 | 1 | 0 | 0 |
| Aureomycin | 4 mg. aureomycin HCl with 2 mg. sodium glycinate in 0.1 cc. of 0.85% NaCl intravenously; killed in 18 hr. | 10 | 0 | 0 | 1 | 5 | 4 |

TABLE 9.—*Effect of Size of Dose of Intravenously Administered Aureomycin, Terramycin, and Chloramphenicol on Livers of Mice*

| Group | Procedure | No. of Mice | Degree of Microscopic Changes in Liver (H and E) | | | | | Degree of Microscopic Changes in Liver (Scarlet Red) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1+ | 2+ | 3+ | 4+ | 0 | 1+ | 2+ | 3+ | 4+ |
| Control | 0.2 cc. NaCl intravenously; killed 18 hr. later | 7 | 1 | 4 | 2 | 0 | 0 | 6 | 1 | 0 | 0 | 0 |
| Terramycin | 75 mg./Kg. in 0.2 cc. NaCl intravenously; killed 18 hr. later | 5 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | 0 |
| | 150 mg./Kg. in 0.2 cc. NaCl intravenously; killed 18 hr. later | 5 | 0 | 0 | 3 | 2 | 0 | 0 | 1 | 4 | 0 | 0 |
| | 250 mg./Kg. in 0.2 cc. NaCl intravenously; killed 18 hr. later | 8 | 0 | 0 | 3 | 4 | 1 | 0 | 1 | 4 | 1 | 2 |
| Aureomycin | 75 mg./Kg. in 0.2 cc. NaCl intravenously; killed 18 hr. later | 5 | 0 | 0 | 1 | 4 | 0 | 0 | 1 | 3 | 1 | 0 |
| | 150 mg./Kg. in 0.2 cc. NaCl intravenously; killed 18 hr. later | 8 | 0 | 1 | 1 | 1 | 5 | 0 | 0 | 0 | 4 | 4 |
| Chloramphenicol | 187 mg./Kg. in 0.01 cc. acetyldimethylamine intravenously; killed 18 hr. later | 7 | 2 | 4 | 0 | 0 | 1 | 2 | 1 | 4 | 0 | 0 |
| | 250 mg./Kg. in 0.01 cc. acetyldimethylamine intravenously; killed 18 hr. later | 8 | 2 | 1 | 2 | 3 | 0 | 7 | 0 | 1 | 0 | 0 |
| | 375 mg./Kg. in 0.01 cc. acetyldimethylamine intravenously; killed 18 hr. later | 2* | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |

* Five animals were given injections in this group; three died within one minute following the injection.

istration. In this experiment where a semiquantitative effect was being sought, fat stains with scarlet red were made in addition to the hematoxylin and eosin stains.

Dogs were used in one experiment, as follows: Needle biopsies of the liver were performed on four dogs. The animals were then given 50 mg. of aureomycin hydrochloride in 10 cc. of isotonic sodium chloride solution daily. One dog died of intraperitoneal hemorrhage after the second injection. The other three dogs received four daily injections of the drug. Needle biopsies of the liver were performed 24 hr. after the fourth injection. In addition, biopsy was performed on one dog three days and again 10 days after the last injection of aureomycin.

RESULTS

In many instances, the livers of the mice which had received aureomycin and terramycin parenterally were pale yellow and, thus, easily differentiated grossly from the livers of the controls. Many of the livers in the treated animals were uniformly pale, while others had areas 3 to 4 mm. in diameter which were yellower than the surrounding tissue. The capsules over such areas, however, were smooth and glistening. Moreover, in many specimens there were punctate white spots that resembled small necroses.

Microscopically, almost all the livers from mice which had received aureomycin parenterally showed vacuolation in the cytoplasm of the hepatic parenchymal cells. The changes, when limited in degree, were located in the center of the lobule. Typical changes are shown in Figures 1, 2, and 3. Fat stains (Fig. 4) indicated

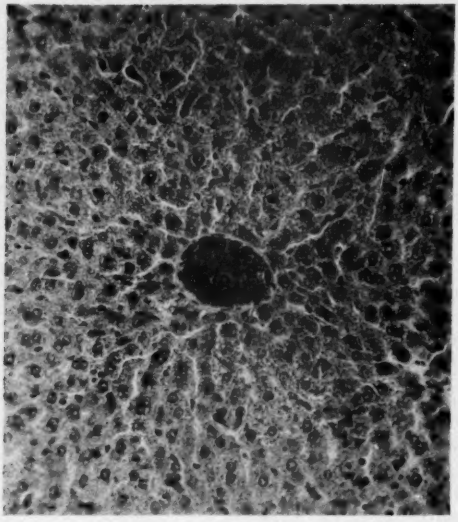

Fig. 1.—Liver of mouse receiving saline solution intraperitoneally. Hematoxylin and eosin; × 200.

that much of the vacuolation was caused by large amounts of lipid material, although glycogen stains indicated that this, too, may have been depleted. In recording the data, we have classified the extent of change from 0 to 4 + on the basis of the degree of involvement of the individual cells and of the liver as a whole, as determined by microscopic examination.

From Table 1 it is clearly seen that, whereas only four among 48 control mice exhibited minimal changes in the liver, the majority (30 among 47) of those receiving aureomycin intraperitoneally showed marked changes and only two showed no change. Moreover, in Table 2 it can be seen that all the animals which received aureomycin had moderate to marked changes, regardless of the presence or absence of sodium glycinate in the solution or of the route of parenteral administration. On

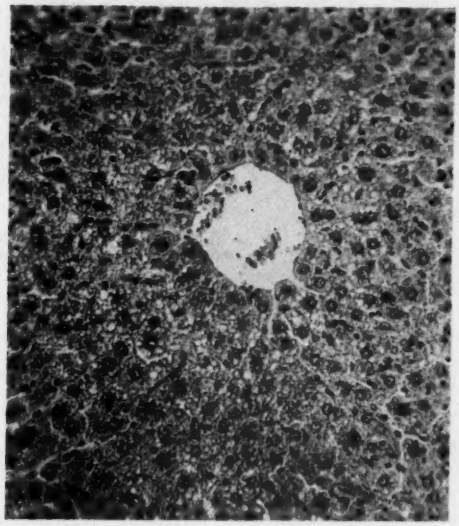

Fig. 2.—Central vacuolation of mouse liver after three intravenous injections of aureomycin. Hematoxylin and eosin; × 200.

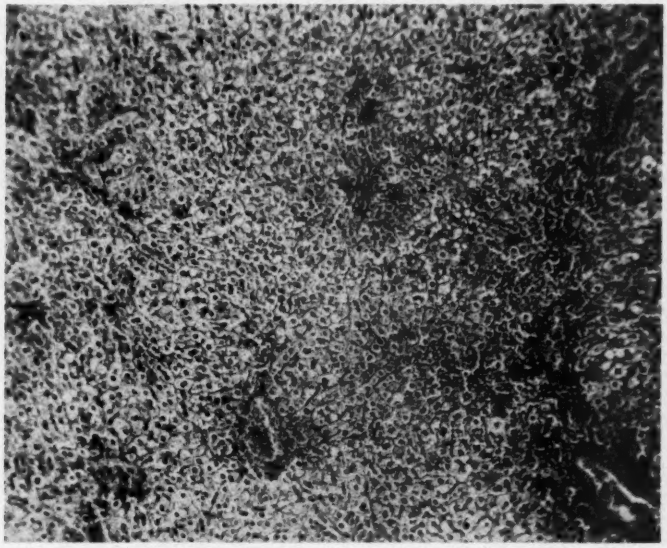

Fig. 3.—Section of liver from mouse which received aureomycin intraperitoneally; × 100.

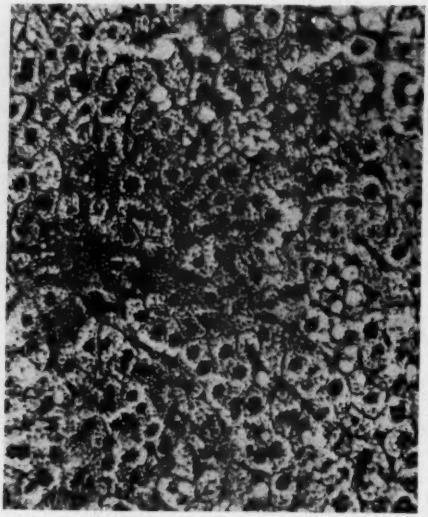

Fig. 4.—Liver of mouse which had received aureomycin intraperitoneally for seven days. Note fragmentation and vacuolation of cytoplasm. Hematoxylin and eosin; × 300.

Fig. 5.—Fat stain (oil red O) showing fat vacuoles in liver of mouse given aureomycin intravenously. Hematoxylin and eosin; × 200.

the other hand, sodium glycinate itself produced only 1 to 2 + changes in the livers of five among 12 animals, and even as strong an irritant as 0.001 M hydrochloric acid given intraperitoneally produced only 1 to 2 + changes in eight of 10 mice. In this experiment also, the livers of the mice in the saline control group showed practically no microscopic change.

The results in the third experiment (Table 3) were conclusive only in that they indicated that 2 to 4 + changes again occurred in all animals, and that such changes started within the first 24 hr. and became progressively worse with the further administration of the drug. All but two among 22 control-fed mice had 0 to 2 + damage.

Several significant facts became apparent in Experiment 4. In the first place, the mice which received aureomycin ate as well as the control animals who were allowed to eat ad libitum. Hence, there was no evidence that any of the mice in the three groups had insufficient food. Moreover, there was no correlation in any of the three groups between the food intake and the appearance of the liver. As shown in Table 4, the livers from all 10 mice which received aureomycin were moderately or markedly involved, while only one among 20 control animals had as much as a 2 + change. Moreover, the fact that the results in the two control groups were essentially identical demonstrates that there was no significant dietary deficiency among the group in which food was restricted.

In the fifth experiment (Table 5) oral administration of either aureomycin or terramycin produced 1 to 2 + changes in about half of the mice, whereas the livers of the other half, and of all the animals which received chloramphenicol, appeared normal. Furthermore, while intraperitoneal aureomycin and terramycin administration consistently produced 3 and 4 + changes, the administration of chloramphenicol or saline solution was followed by 1 + changes in less than half of the mice.

The sixth experiment confirmed the fact that even in large doses aureomycin caused fewer changes when given by mouth. However, one 3 + reaction and four 2 + reactions occurred among 11 animals so treated. Terramycin in large doses intraperitoneally caused little more damage than in the controls, although the rapid death which occurred in most animals in this group made the result difficult to interpret. Chloramphenicol, even when it was administered intraperitoneally in large doses, produced only mild changes.

Heat-inactivated aureomycin produced approximately as extensive a change as active aureomycin.

As shown in Table 7, intravenous administration of aureomycin produced 3 or 4 + changes in the livers of eight of 10 animals, whereas only one 3 + change was produced among 10 animals which had a similar dietary intake without aureomycin. However, as we mentioned previously, the tails of the animals in this group which were treated with aureomycin showed considerable tissue damage from the injections.

Table 8 illustrates that when a dose of aureomycin in the lower lethal range [2] was used marked changes occurred in almost all livers within 18 hr., whereas the control livers were not affected by a similar injection of saline solution. In these animals no evidence of tissue irritation was to be seen.

It can be seen in Table 9 that the action of aureomycin was more pronounced than that of the other antibiotics and that the effect depended in part on the dose used. On the other hand, there were definite changes after the administration of

terramycin, and this effect was definitely proportional to the dose administered. The results with chloramphenicol administration were much less striking. In the higher dose range there were a few livers which appeared to have some changes. Since the dose of 375 mg. per kilogram killed three of five mice instantaneously, there were only two animals in this group.

The results of the single experiment performed on dogs were as follows: Three of the dogs survived four daily intravenous doses of aureomycin. All three showed distinct changes in the liver, consisting of vacuolation limited to the central half of each lobule. The biopsy specimen of the liver of one of the dogs three days after the last injection of aureomycin showed a decrease in the degree of vacuolation. One week later a needle biopsy specimen of the liver revealed restoration to essentially normal structure.

## COMMENT

From the experiments performed on mice it is clear that the parenteral administration of aureomycin and terramycin in relatively large single daily injections subcutaneously, intraperitoneally, or intravenously is capable of producing gross and microscopic changes in the livers of mice. Similar changes have been partially produced in dogs.

Several questions immediately arise: 1. What is the mechanism of production of the changes? 2. What is the significance of the changes? 3. What further studies should be done?

The results of the present study throw little light on the mechanism of the production of the changes. Since aureomycin that had been almost completely inactivated by incubation at 37 C. was as effective as the unaltered drug, the hepatic action does not appear to be related to the antibacterial activity. Furthermore, there is no evidence that the administration of aureomycin caused the mice to eat less, nor did a restriction in the food intake of the control animals produce so extensive a change. Also, the irritant properties of the antibiotic on the tissues do not account for the changes, since similar changes were not observed with administration of unbuffered hydrochloric acid. In addition, the changes could be produced readily without concomitant damage to the tissues by the injection of large amounts of aureomycin in a single dose directly into the vein. It is still possible, however, that the changes seen are nonspecific and represent the summation of the effects of the trauma to the tissues, acidity of the compound, and the like. Certain facts, however, suggest that the effect may be a direct action of aureomycin and terramycin on the liver. First, regardless of the route used for injection in the mice and, therefore, independent of the degree of trauma, the changes in the liver were quite similar. Even when the oral route was used, changes which were qualitatively the same, although quantitatively less, occurred in the liver if large doses were employed. In addition, the excellent correlation of extent of change with amount of drug administered argues for the effect to be an inherent property of the drug. Moreover, comparable changes were present in the livers of the dogs who received the aureomycin by the least traumatic route.

In the absence of a conclusive answer on the mechanism of the change, it is difficult to evaluate the significance of the observations. Since the survival rate among the mice was high and since there was no evidence of jaundice in the viscera, the changes within the cells may not be correlated with serious alteration of function. However, the effect does become more pronounced as a lethal dose is approached.

Because there was a striking similarity in the microscopic picture in the livers of the mice and in the livers of patients who exhibited signs of hepatic dysfunction while receiving aureomycin,[1] we feel that the observed changes are probably of pathologic significance. It is possible, however, that the morphologic similarity is fortuitous and not related to a common toxic effect. This question would be more definitely answered if the changes in mice were clearly shown to be caused by the antibiotic per se and not a result of secondary factors.

The results obtained with chloramphenicol administration are not conclusive. Since there were suggestive changes from single large doses administered intravenously, the drug should be investigated further with large repeated daily doses to see if it has the same potential effect but to a less marked degree.

From the above discussion the need for further study is obvious. Experiments must be performed to differentiate the possible metabolic, nutritional, and irritant effects. Studies should also be done in an animal, such as the dog, in order to relate results of tests of liver function to microscopic observations in serial biopsy specimens. If the changes then appear to be due to a toxic effect on the liver, it would be appropriate to study the reversibility of the changes, especially in regard to whether special dietary measures and/or vitamin therapy would reverse such changes. The fragmentary observations on the dogs suggest that the changes are rapidly reversible.

## SUMMARY

Aureomycin hydrochloride, in doses of 75 to 150 mg. per kilogram of body weight buffered with sodium glycinate and dissolved in isotonic sodium chloride solution, given intravenously, intraperitoneally, or subcutaneously in a single injection daily produced microscopic changes in the livers of mice.

Similar changes were not produced by intraperitoneal injection of sodium glycinate alone or by dilute hydrochloric acid.

Limitation of food intake by the mice receiving aureomycin did not account for the changes.

Only minimal hepatic changes were produced by large doses (320 to 400 mg. per kilogram of body weight) of aureomycin given by mouth.

Inactivated aureomycin was as effective in producing the observed changes as aureomycin itself.

The effect of terramycin was similar to that of aureomycin, although less marked. No definite answer could be obtained concerning whether chloramphenicol caused similar changes because it was necessary to raise the dose of this drug to levels in the lethal range before any of the animals showed changes in the liver.

Similar microscopic changes were seen in dogs on which serial liver biopsies were done after intravenous administration of aureomycin.

It is felt that the changes, because of their constancy of and their similarity to changes seen in jaundiced patients receiving large doses of aureomycin by the intravenous and oral routes combined, may have clinical and pathologic significance. It is emphasized, however, that the doses of aureomycin or terramycin employed throughout these experiments are much higher than those which are recommended for the treatment of human infections. As suggested elsewhere,[1] we recommend that not more than 2 gm. of aureomycin be administered a day by the intravenous route and not more than 1 gm. a day when adjuvant oral therapy is employed. Doses of terramycin would presumably be the same.

# CLINICAL EVALUATION OF 4-HYDROXYCOUMARIN ANTICOAGULANT NO. 63

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AND
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THE FIRST synthesis of the 4-hydroxycoumarin anticoagulant No. 63, a compound chemically related to bishydroxycoumarin (dicumarol®; 3,3'-methylenebis [4-hydroxycoumarin]), was by Dr. Myoshi Ikawa in 1942 in Dr. Karl Paul Link's laboratory.[1] It is a colorless and stable product which is readily prepared in pure form, and its solubility in water is of the order of 1/100 that of bishydroxycoumarin. Chemically anticoagulant No. 63 is 2-methyl-2-methoxy-4-phenyl-5-oxodihydropyrano-(3,2-c) (1) benzopyran. In work done by Scheel [2] in the mouse, rat, rabbit, and dog, attention was given not only to activity per se, as reflected by the prolongation of the 12.5% plasma-prothrombin time, but also to the time and site of appearance of visible hemorrhage and the survival time resulting from continued administration of various coumarin anticoagulants. He concluded that 4-hydroxycoumarin anticoagulant No. 63 was a strong competitor, if not an agent superior, to bishydroxycoumarin. In dogs and in human beings this anticoagulant was more potent on a milligram-per-kilogram basis than bishydroxycoumarin; when given to dogs over a period of days the degree of anticoagulant action was greater and the duration of effect was longer without the appearance of hemorrhagic manifestations.

The initial clinical observations on this compound were made by Battle, Capps, Orth, and Meyer,[3] who demonstrated that a single oral dose of 2 mg. per kilogram of body weight was capable of producing an adequate hypoprothrombinemia within 24 to 48 hr. The latent period before demonstrable effect from this drug was approximately 24 hr., but a therapeutic level was usually more rapidly attained with

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This study was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

1. Ikawa, M.; Stahmann, M. A., and Link, K. P.: Studies on 4-Hydroxycoumarins: The Condensation of Alpha, Beta-Unsaturated Ketones with 4-Hydroxycoumarin, J. Am. Chem. Soc. **66**:902 (June) 1944.

2. Scheel, L. D.: Studies on the Anticoagulant Dicumarol and Other 4-Hydroxycoumarins, Doctoral Dissertation, University of Wisconsin, University of Wisconsin Press, 1949. Scheel, L. D.; Wru, D., and Link, K. P.: 4-Hydroxycoumarin Anticoagulants, read before the 16th Meeting of the American Chemical Society, Sept. 18-23, 1949, abstract of papers, p. 76.

3. Battle, W. D.; Capps, R. T.; Orth, O. S., and Meyer, O. O.: The Effects of 4-Hydroxycoumarin Anticoagulant No. 63 upon the Prothrombin Time in Dogs and Human Beings, J. Lab. & Clin. Med. **35**:8 (Jan.) 1950.

compound No. 63 than with bishydroxycoumarin. The prothrombin time returned to the pretreatment level in 12 or 13 trials with a single dose in an average of eight days, with extremes of four and 14 days. In these patients there were no demonstrable changes in the complete blood count, urinary findings, or results of studies of hepatic function.

Further observations of Rotter and Meyer [4] have emphasized the utility of this anticoagulant in man and particularly the ease of maintaining a stable level of hypoprothrombinemia with its administration. These studies were extended in an attempt to determine whether or not compound No. 63 would satisfy the criteria for an ideal anticoagulant; that is, one that is rapidly absorbed and uniformly metabolized by different subjects, with prompt therapeutic action and without toxic side effects.[5] Here our aim has been to find an anticoagulant which produced hypoprothrombinemia of such regular degree and stability that the frequency of prothrombin-time determinations could be lessened.

## MATERIAL AND PROCEDURES

*Material.*—This is a report on 130 trials in 124 patients in the State of Wisconsin General Hospital who were treated with 4-hydroxycoumarin anticoagulant No. 63. Fifty-three of these patients had no evidence of thromboembolic disease, and they were given the drug for the purpose of establishing proper dosage and for testing of antidotes. The diagnoses included rheumatoid arthritis, psychoneurosis, rheumatic heart disease, Parkinsonism, carcinoma, diabetes, asthma, Hodgkin's disease, osteoarthritis, nontoxic goiter, sinusitis, and cholelithiasis. Seventy-one additional patients in whom it was felt that anticoagulant therapy was specifically indicated received this preparation. The diagnoses in this group included myocardial infarction, thrombophlebitis, phlebothrombosis, pulmonary infarction, cerebral thrombosis and embolism, and peripheral arterial embolism. The sexes were represented in about equal numbers.

*Procedures.*—Determinations of prothrombin times were performed on 100% plasma by the one-stage method of Quick [6] or its Link-Shapiro modification [7] using difco® thromboplastin (rabbit brain). The optimum calcium concentration recommended by Stewart and Pohle [8] was employed. For each patient all prothrombin studies were performed by the same technicians daily at approximately the same time. Control prothrombin times varied between 11 and 13 sec., and the results were reported in percentages of normal. An attempt was made to keep the values between 20 and 40% of normal as a probable satisfactory therapeutic range. During these studies the original observations,[5] which indicated a lack of toxicity, were further confirmed by complete blood counts, urinalyses, and hepatic function tests. These tests included cephalin-cholesterol, thymol-turbidity, icterus-index, serum-bilirubin, urinary-urobilinogen, and serum-albumin and globulin determinations.

In all cases a base-line prothrombin time was determined, and no anticoagulant was administered unless the value was 70% or more. The initial dose was 2 mg. per kilogram of body

4. Rotter, R., and Meyer, O. O.: Clinical Evaluation of 4-Hydroxycoumarin Anticoagulant No. 63, Proc. Central Soc. Clin. Res. **23**:89 (Nov.) 1950; J. Lab. & Clin. Med. **36**:981 (Dec.) 1950.

5. Shapiro, S., and Weiner, M.: Dicumarol Therapy, J. M. Soc. New Jersey, **48**:52 (Feb.) 1951.

6. Quick, A. J.: The Nature of the Bleeding in Jaundice, J. A. M. A. **110**:1658 (May 14) 1938.

7. Campbell, H. A.; Smith, W. K.; Roberts, W. L., and Link, K. P.: Studies on the Hemorrhagic Sweet Clover Disease: II. The Bioassay of Hemorrhagic Concentrates by Following the Prothrombin Level in the Plasma of Rabbit Blood, J. Biol. Chem. **138**:1 (March) 1941. Shapiro, S.; Sherwin, B.; Redish, M., and Campbell, H. A.: Prothrombin Estimation: A Procedure and Clinical Interpretation, Proc. Soc. Exper. Biol. & Med. **50**:85 (May) 1942.

8. Stewart, J. K., and Pohle, F. J.: Effect of Calcium in Quantitative Determination of Prothrombin, Proc. Soc. Exper. Biol. & Med. **39**:532 (Dec.) 1938.

weight, and the drug was given to the nearest 25 mg. No anticoagulant was administered on the second day. Subsequent dosage was governed by the prothrombin time, and the average daily maintenance dose was 0.5 to 1.0 mg. per kilogram of body weight. Usually, as with bishydroxycoumarin, no anticoagulant was administered if the prothrombin percentage was in the lower therapeutic range and decreasing or was below 20%.

The dosage schedule adopted for the administration of this drug was usually as follows:

1. If the prothrombin for a specified day was between 20 and 40%, 25 mg. of the anticoagulant was given.

2. If the prothrombin was below 20% of normal, no further anticoagulant was administered until the value rose to a point within the therapeutic range.

3. If the prothrombin was over 40%, 50 mg. of the anticoagulant was given.

## RESULTS

*Onset of Effect.*—In 124 of the 130 trials with this preparation, the drug was administered initially; while in six cases, the patients were changed from bishydroxycoumarin to compound No. 63 treatment after a therapeutic level had been attained. As had been noted previously, there was a latent period of approximately 24 hr. before hypoprothrombinemic effects became apparent. In 20 (16%) of the instances in which the drug was used initially, the therapeutic range was actually reached in 24 hr., and in an additional 77 (62%) the therapeutic level was attained within 48 hr. Thus, a satisfactory therapeutic response was present in 78% of the patients within 48 hr.

More anticoagulant was ordinarily given if a prothrombin value at least as low as 40% did not occur within 48 hr. An additional 14 patients (11%) were within therapeutic range in 72 hr. Still more anticoagulant brought another five patients (4%) to a therapeutic level in 96 hr. Five patients were considered to be quite resistant to the anticoagulant and required 120 to 168 hr. to reach a satisfactory level. Two patients failed to attain the therapeutic range after the initial dose, and no further anticoagulant was given to them.

*Maintenance of Effect.*—In general, these studies confirm the previous observations [4] that anticoagulant No. 63 is capable of maintaining a satisfactory prothrombin level with a relatively fixed dose of the drug. Figure 1 depicts a typical response to a single dose of this compound, and Figure 2 indicates that the control can be well maintained with a constant dose of the anticoagulant. This patient was kept within the narrow range of 22 to 30% for 20 days while receiving a daily dose of 25 mg. of compound No. 63. Other patients were controlled equally well after a dosage pattern had been established, although the amount of anticoagulant required varied from 25 to 50 mg. every day, every other day, or every third day. In the treatment of thromboembolic disorders, the required schedule of drug administration became apparent within the first or second week of therapy, and maintenance was no more difficult in these patients than in those without thrombotic disease.

Only one attempt was made to maintain a patient as an outpatient with the use of compound No. 63. This person, a 36-yr.-old university student, was hospitalized because of the development of thrombophlebitis in his left leg and was treated with this anticoagulant. After 10 days of hospitalization, the patient was discharged and treatment with the drug was continued, since there was evidence of persistent swelling and induration in his leg. During the ensuing 14 weeks the patient took

25 mg. of compound No. 63 six days out of seven and had a prothrombin-time determination two or three times a week. Except for four values of more than 40%, the patient's prothrombin remained within the therapeutic range, 20 to 40%, with no evidence of bleeding.

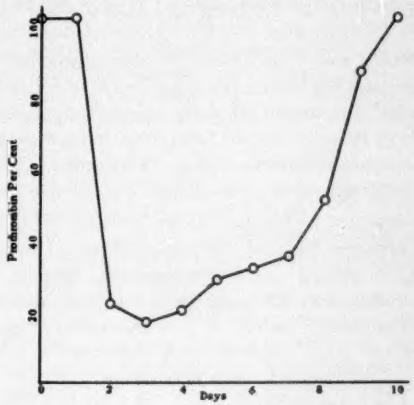


Fig. 1.—The effect of a single oral dose (2 mg. per kilogram of body weight) of compound No. 63 in a 69-yr.-old white woman with rheumatoid arthritis.

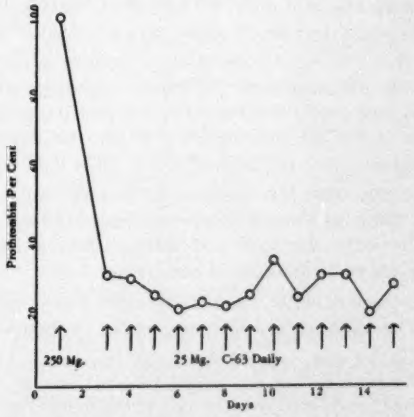


Fig. 2.—Therapeutic effect of multiple doses of compound No. 63 in a white woman with myocardial infarction. The patient was maintained at this prothrombin level for a total of 20 days, with administration of 25 mg. of compound No. 63 each day.

*Hyperreactors.*—The initial dose of 2 mg. per kilogram of body weight proved to be distinctly excessive for only two of the 124 patients. In one the prothrombin fell to 16% within 48 hr. and remained below the therapeutic range for 13 days. In another patient the prothrombin reached a level of 16% 72 hr. after the initial dose was given, and it remained below 20% during the ensuing six days. Sub-

sequently this patient's prothrombin level was maintained with a dose of 25 mg. given six days out of seven. In two additional patients the values dropped below the therapeutic range within 48 hr. and remained so for three days.

Seven other patients maintained a prothrombin value below 20% for five to 10 successive days at various stages of their treatment. The reason for this was not obvious. However, gross bleeding was evident in only one of these patients. (See Case 1 below.)

*Duration of Therapy and Total Dosage of Anticoagulant.*—At the time of this writing, a total of 64 patients had completed a course of anticoagulant therapy that exceeded seven days. Twenty-one of these received treatment from one to two weeks; 17 from two to three weeks; 10 from three to four weeks; six from four to five weeks, and 10 for more than five weeks. Three patients received anticoagulant therapy for 10 to 14 weeks, with no evidence of toxic side effects.

Thirty patients received a total of 500 mg. or more of this anticoagulant. Of these, 15 required between 500 and 750 mg., and an additional nine were given between 750 and 1,000 mg. Five patients received a total of 1,000 mg. or more. The maximum total dose was 2,550 mg., and this was administered to a patient with myocardial infarction throughout a 10-week period. Again, no toxic effects were demonstrable.

## TOXIC MANIFESTATIONS

There were no adverse gastrointestinal symptoms from the oral use of anticoagulant No. 63. A common procedure for detecting early hemorrhagic manifestations in patients receiving anticoagulants is the microscopic examination of the urine for the appearance of erythrocytes. Urinalyses were performed in each of a series of 16 patients when their prothrombin values fell below 20% of normal after the use of compound No. 63. Gross urinary bleeding occurred in none of the patients, and in only one was there microscopic evidence of hematuria. Seven erythrocytes per high-power field appeared in the centrifuged urine of this patient, whose prothrombin value had been below 20% for five days. This incidence of microscopic hematuria in 6% of patients is less than half that noted by Meyer, Bingham, and Axelrod [9] with the use of bishydroxycoumarin. Battle and his coworkers [5] reported that large doses of compound No. 63 did not produce the vascular damage induced by large doses of bishydroxycoumarin, and this may be the explanation for the lowered incidence of hematuria.

Gross bleeding attributable to the anticoagulant was noted in four of the 124 treated patients. The following are brief summaries of these cases.

CASE 1.—This 67-yr.-old white man had been under observation at the State of Wisconsin General Hospital for three years and was treated for giant follicular lymphoma with several courses of high-voltage x-ray therapy. On his final admission he complained chiefly of shortness of breath, cough, weakness, and loss of appetite. His thrombocyte (platelet) count was 74,000 per cubic millimeter. Therapy with anticoagulant No. 63 was initiated because of the development of phlebothrombosis in the right femoral vein. When the prothrombin fell to 6%, rectal and nasal bleeding occurred. The patient was given 500 mg. of vitamin $K_1$ orally and was transfused with 500 cc. of whole blood. Twenty hours later the prothrombin was 40%, and the bleeding manifestation gradually ceased as the prothrombin continued to rise to 62%. The patient died eight days after the vitamin $K_1$ had been given, and a necropsy revealed the

9. Meyer, O. O.; Bingham, J. B., and Axelrod, V. H.: Studies on the Hemorrhagic Agent, 3,3'-Methylenebis (4-Hydroxycoumarin), Am. J. M. Sc. **204**:11 (July) 1942.

presence of extensive lymphoblastoma, pulmonary thrombosis, lung abscess, and thrombosis of the distal portion of the inferior vena cava. There was no evidence of recent or old hemorrhage.

*Comment.*—Probably poor judgment was used in this instance in giving an anticoagulant in the presence of thrombocytopenia of this degree.

CASE 2.—The chief complaint of this 64-yr.-old white diabetic woman was swelling of the tongue and neck. Physical examination revealed the presence of acute left maxillary sinusitis, with orbital and submaxillary cellulitis. Anticoagulant therapy was initiated as prophylaxis against possible cavernous sinus thrombosis. When the prothrombin fell to 11% of normal, large ecchymoses appeared over the right flank and left hip after vigorous massage by a member of the nursing staff. The combined use of blood transfusions and a water-soluble vitamin-K preparation resulted in the gradual return of the prothrombin to 100% in five days. However, these ecchymotic areas became indurated, necrotic, and ulcerated, requiring débridement and plastic repair.

*Comment.*—The trauma of the massage, we believe, was directly responsible for the ecchymoses, although, admittedly, bleeding probably would not have occurred, or been so severe, had the excessive hypoprothrombinemia not existed.

CASE 3.—A 70-yr.-old white woman presented the chief complaint of the presence of pain in the right upper portion of the abdomen for 15 yr. Physical examination, laboratory tests, and roentgenologic studies disclosed the presence of a chronic duodenal ulcer, arteriosclerotic heart disease, senile osteoporosis, Parkinsonism, and chronic cervicitis. Two days after a biopsy of the cervix, the patient was given anticoagulant No. 63. When her prothrombin fell to 15% of normal four days later, the patient showed a moderate amount of vaginal bleeding, with the passage of a clot. Examination revealed the presence of slight oozing at the biopsy site where a catgut suture had sloughed. After cauterization of the site with silver nitrate, the bleeding was controlled. One gram of vitamin $K_1$ was administered orally after the cauterization, and within 20 hr. the prothrombin was up to 77%, with no further evidence of bleeding.

*Comment.*—Since the prothrombin was reduced to 15%, one is justified in concluding that this probably played at least some role, and possibly a major role, in the development of the bleeding in this patient.

CASE 4.—A 63-yr.-old white man was admitted to the hospital because of recurrent pain and swelling in the left lower leg. He had previously been treated for bronchial asthma and arteriosclerotic heart disease. In addition to the findings of emphysema and asthmatic rales, the skin of the lower left leg was observed to be tense, shiny, red, and warm, with evidence of ropelike veins above the indurated area. A diagnosis of thrombophlebitis was made, and compound No. 63 was administered. Two days later, when the prothrombin fell to 23%, the patient had a subconjunctival hemorrhage measuring 0.5 by 1.0 cm. This was not considered to be a contraindication to further anticoagulant therapy; despite the maintenance of the prothrombin within the therapeutic range, the hemorrhage rapidly absorbed. No further hemorrhagic manifestations were observed during the ensuing three weeks of therapy.

*Comment.*—This must be looked upon as a minor hemorrhagic episode, and its occurrence did not serve as a deterrent to further anticoagulant therapy.

### EFFECT OF VARIOUS CORRECTIVES

Various agents were tried in an attempt to reverse the hypoprothrombinemia induced by compound No. 63. These included blood transfusions, two water-soluble vitamin-K preparations (synkayvite® [10] [tetrasodium 2-methyl-1,4 naphthohydroquinone diphosphoric ester, a synthetic vitamin-K analogue] and menadione sodium bisulfite [hykinone®]), vitamin $K_1$ [11] (2-methyl-3-phytyl-1,4-naphthoquinone), and vitamin $K_1$ oxide.[12] In some cases, an attempt was made to determine whether or

10. The synkayvite® used in this study was supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

11. The vitamin $K_1$ used in this study was supplied by Merck & Company, Inc., Rahway, N. J.

12. Dr. R. K. Richards, of Abbott Laboratories, North Chicago, Ill., supplied the vitamin $K_1$ oxide used in this study.

not the antidote was directly antagonistic to the anticoagulant by administering the two preparations simultaneously; in other cases, the antidote was tested after the hypoprothrombinemia had been induced.

*Blood Transfusions.*—A total of 11 blood transfusions was administered to eight patients who had hypoprothrombinemia induced by treatment with compound No. 63. Five of these patients received a single transfusion of 500 cc., and three were each given 500 cc. on each of two successive days. The results are charted in Table 1, exclusive of those in the two patients that received a vitamin-K preparation as well. Two of the patients who received 500 cc. of fresh blood when the prothrombin was 17 and 13%, respectively, failed to show any alteration four hours later. Twenty hours afterward the prothrombin values were 19 and 28%, respectively, and each patient was then given another fresh-blood transfusion. Four hours after the second transfusion the prothrombin values were 24 and 29%, and 20 hr. later they actually fell to 21 and 22%, respectively. Another patient with a pro-

TABLE 1.—*Effect of Blood Transfusions on Hypoprothrombinemia Induced by Administration of Compound No. 63*

| Patient No. | No. of Transfusions | Prothrombin Percentages | | |
|---|---|---|---|---|
| | | Before Transfusion | After Transfusion | |
| | | | 4 Hr. | 20 Hr. |
| 1 | 2 | 17 | 17 | 19 |
| | | 19 | 24 | 21 |
| 2 | 2 | 13 | 13 | 28 |
| | | 28 | 29 | 22 |
| 3 | 1 | 30 | 27 | 28 |
| 4 | 1 | 35 | 47 | 47 |
| 5 | 1 | 55 | 69 | 76 |
| 6 | 1* | 27 | 34 | 37 |
| Averages | | 28 | 32 | 35 |

* Twenty-five milligrams of compound No. 63 was given simultaneously.

thrombin of 30% received a fresh-blood transfusion of 500 cc., and four and 20 hr. later the prothrombin percentages were 27 and 28, respectively.

Two other patients receiving compound No. 63 were given 500 cc. transfusions of 24-hr. bank blood; prothrombin-time determinations were made after 1, 2, 4, and 20 hours. In one patient a prothrombin level of 35% changed to 41% in one hour, was the same two hours later, and increased to 47% after four hours. There was no further change on the following day. The other patient failed to reach the therapeutic level within 48 hr. after administration of the anticoagulant, and the prothrombin percentage responded somewhat better to the blood transfusion. The prothrombin value increased from 55 to 69% within one hour and maintained this level each hour for four hours. On the following day, the prothrombin was 76% of normal. Still another patient was given a transfusion of 24-hr. bank blood, but in this case a maintenance dose of anticoagulant was given simultaneously. The prothrombin value changed from 27 to 34% in four hours and to 37% in 20 hr. In two other patients a vitamin-K preparation was administered along with the blood transfusions; so the effect of the blood per se could not be evaluated. The combination of 50 mg. of water-soluble vitamin K given intravenously and a transfusion of 500 cc. of blood to one patient was capable of increasing the prothrombin

from 11 to 35% in 20 hr. This therapy was repeated, and 44 hr. later the prothrombin was 67%. In the other patient with a prothrombin level of 6%, 500 mg. of vitamin $K_1$ was administered orally and four hours later the prothrombin value was 13%. The patient was then given a transfusion of 500 cc. of fresh blood, and in 20 hr. the prothrombin increased to 40%.

With the exclusion of the three instances in which the vitamin-K preparation was given along with the blood transfusions, the average value of the prothrombin before the transfusions were administered was 29%. The average values four and 20 hr. later were 32 and 35%, respectively. These changes were considered to be insignificant.

*Water-Soluble Vitamin-K Preparations.*—When synkayvite® was administered to six patients with hypoprothrombinemia induced by compound No. 63, the effect produced was always slight. The results are shown in Table 2. A dose of 75 mg. of synkayvite® administered intravenously was capable of producing an increase in

TABLE 2.—*Effect of Intravenous Use of Synkayvite® on Hypoprothrombinemia Induced by Treatment with Compound No. 63*

| Patient No. | No. of 75-Mg. Doses | Prothrombin Percentages | |
|---|---|---|---|
| | | Before Synkayvite ® | After Synkayvite ® (20 Hr.) |
| 1 | 1 | 20 | 30 |
| 2 | 1 | 60 | 60 |
| 3 | 1 | 21 | 28 |
| | 3* | 28 | 31 |
| 4 | 6 | 23 | 38 |
| 5† | 1 | 24 | 38 |
| | 1 | 38 | 61 |
| 6† | 12* | 25 | 35 |
| Averages..... | | 30 | 40 |

* Twenty-five milligrams of compound No. 63 was given simultaneously.
† Synkayvite® was administered intramuscularly to Patient 5 and orally to Patient 6.

the prothrombin from 20 to 30% in 20 hr. in one patient. No change was produced in another patient in four and in 20 hr. with the same dose when the initial prothrombin was 60%. In a third patient the prothrombin value increased from 21 to 28% 20 hr. after 75 mg. of synkayvite® had been given intravenously. An additional three doses of the preparation were administered to this patient at four-hour intervals, along with a single dose of 25 mg. of the anticoagulant. Four hours after the last dose of synkayvite® the prothrombin was 33%, and 16 hr. later it had dropped to 31%. A total of 450 mg. of synkayvite® given to another patient in six divided doses over a 24-hr. period produced a change from 23 to 38%. When this preparation was administered intramuscularly to a fifth patient, a dose of 75 mg. produced an increase from 24 to 38% in 20 hr. and to 61% 20 hr. after still another dose. A total of 900 mg. of synkayvite® was given orally in two divided doses to another patient, along with 25 mg. of compound No. 63, and a prothrombin value of 25% rose to 35% 20 hr. after the last dose of synkayvite® was given.

A second water-soluble vitamin-K preparation, menadione sodium bisulfite, was administered to six patients in single or multiple intravenous doses of 72 mg., and these results are charted in Table 3. In one patient a single dose produced a change from 30 to 60% in 20 hr. Administration of 72 mg. of menadione sodium bisulfite

failed to alter significantly a prolonged prothrombin time in another patient, the value changing from 6 to 12% of normal in 20 hr. A prothrombin level of 33% rose to 40% in four hours and to 52% in 20 hr. after 72 mg. of this vitamin-K preparation was administered intravenously to a third patient. Three intravenous doses of menadione sodium bisulfite were given to another patient at four-hour intervals, and 20 hr. after the first dose there was a change in the prothrombin from 27 to 62%. A similar regimen in a fifth patient increased the prothrombin from 30 to 45% in 20 hr. No change was noted in a patient with a prothrombin percentage of 23 when a maintenance dose of the anticoagulant was administered, along with a total of 216 mg. of menadione sodium bisulfite intravenously.

Thus, it appears that the usual effects of the water-soluble vitamin-K preparations on the hypoprothrombinemia induced by compound No. 63 were not very marked. With synkayvite®, the average value of the prothrombin before the antidote was administered was 30%, and 20 hr. later the average prothrombin was but 40%. Menadione sodium bisulfite proved to be somewhat better under the conditions

TABLE 3.—*Effect of Intravenous Use of Menadione Sodium Bisulfite (Hykinone®) on Hypoprothrombinemia Induced by Treatment with Compound No. 63*

| Patient No. | No. of 72-Mg. Doses | Prothrombin Percentages | |
|---|---|---|---|
| | | Before Hykinone® | After Hykinone® (20 Hr.) |
| 1 | 1 | 30 | 60 |
| 2 | 1 | 6 | 12 |
| 3 | 1 | 30 | 52 |
| 4 | 3 | 27 | 62 |
| 5 | 3 | 30 | 45 |
| 6 | 3* | 23 | 23 |
| Averages..... | | 25 | 42 |

* Twenty-five milligrams of compound No. 63 was given simultaneously.

in which it was used, but its effects were not striking. From an average value of 25% in the six instances, there was an increase to 42% 20 hr. later. Our impression is that, although a slight increase in the plasma prothrombin occurs after vitamin-K administration or transfusion of blood, these are not potent antidotes for hypoprothrombinemia induced by compound No. 63.

*Vitamin $K_1$.*—This substance is a viscous amber-colored oil supplied in 1-gm. ampuls. This product was administered orally in all instances, and in most patients was given undiluted in gelatin capsules. Initially, 1-gm. doses were used, but later studies were performed using smaller quantities. A total of 21 patients received vitamin $K_1$ in an attempt to demonstrate its effect on the hypoprothrombinemia induced by compound No. 63. One gram was administered to each of 14 patients, seven of whom were given maintenance doses of anticoagulant No. 63 simultaneously. The results charted in Table 4 demonstrate that when the vitamin $K_1$ was administered alone to each of seven patients the average change in the prothrombin was from 20 to 70% in 20 hr. A significant feature is the fact that the initial value was below the therapeutic range in five of the seven patients, but 20 hr. after the vitamin $K_1$ was given all the values were well above the therapeutic range and at a level where bleeding would be quite unusual. When a maintenance dose of compound No. 63 was given along with the tested antidote, there was still

a significant alteration of the prothrombin time. Thus, in 20 hr. an average increase from 27 to 63% occurred, and all the post-treatment values were more than 45%.

Seven additional patients received smaller doses of vitamin $K_1$ dissolved in liquid petrolatum, and the results are charted in Table 5. Quantities varying from

TABLE 4.—*Effect of 1 Gram of Vitamin $K_1$ Given Orally on Hypoprothrombinemia Induced by Treatment with Compound No. 63*

| | Prothrombin Percentages | |
|---|---|---|
| Patient No. | Before Vitamin $K_1$ | After Vitamin $K_1$ (20 Hr.) |
| 1 | 12 | 52 |
| 2 | 17 | 62 |
| 3 | 15 | 77 |
| 4 | 9 | 52 |
| 5 | 16 | 78 |
| 6 | 28 | 100 |
| 7 | 47 | 68 |
| Averages..... | 20 | 70 |

| | Prothrombin Percentages | |
|---|---|---|
| Patient No. | Before Vitamin $K_1$ | After Vitamin $K_1$ and 25 Mg. of Compound No. 63 (20 Hr.) |
| 1 | 30 | 60 |
| 2 | 23 | 73 |
| 3 | 23 | 78 |
| 4 | 22 | 52 |
| 5 | 21 | 58 |
| 6 | 39 | 75 |
| 7 | 32 | 46 |
| Averages..... | 27 | 63 |

TABLE 5.—*Effect of Smaller Oral Doses of Vitamin $K_1$ on Hypoprothrombinemia Induced by Treatment with Compound No. 63*

| | | Prothrombin Percentages | |
|---|---|---|---|
| Patient No. | Vitamin $K_1$ Dose, Mg. | Before Vitamin $K_1$ | After Vitamin $K_1$ (20 Hr.) |
| 1 | 200 | 35 | 80 |
| 2 | 500* | 6 | 40 |
| 3 | 200 | 22 | 35 |
| 4 | 200 | 35 | 62 |
| 5 | 250 | 14 | 100 |
| 6 | 350† | 22 | 52 |
| 7 | 250† | 25 | 79 |
| Averages..... | | 23 | 64 |

* A 500-cc. transfusion of fresh blood was administered with the vitamin $K_1$.
† Patients 6 and 7 were given 25 mg. of compound No. 63 in conjunction with the vitamin $K_1$.

200 to 500 mg. proved very effective in raising the prothrombin percentage in all but one instance, and here the change was from 22 to 35%. In two patients these doses of vitamin $K_1$ produced an average change of prothrombin from 23 to 65%, even though maintenance anticoagulant therapy was simultaneously continued. Patient 5 demonstrated that the smaller doses of vitamin $K_1$ are at times capable of exercising a profound effect on the prothrombin value, producing an increase

from 14 to 100% in 20 hr. In a series of nine patients in whom prothrombin levels were determined four hours after vitamin $K_1$ was given, the average increase in the value was from 24 to 33%.

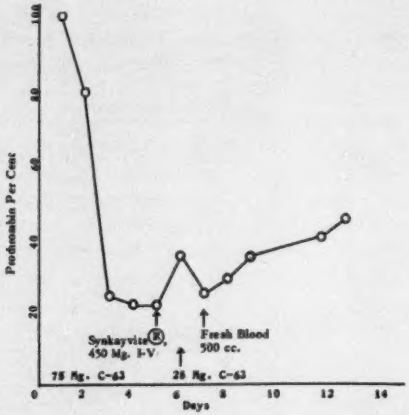


Fig. 3.—A demonstration of the relatively insignificant effects of administration of water-soluble vitamin-K analogue (synkayvite®) and blood transfusions in reversing the hypoprothrombinemia induced by treatment with compound No. 63.

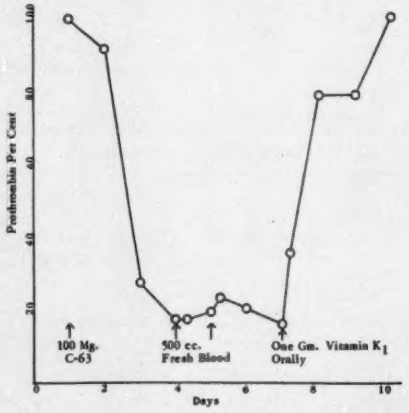


Fig. 4.—A comparison of the ineffectiveness of transfusions of fresh blood and marked effectiveness of administration of vitamin $K_1$ in correcting the hypoprothrombinemia induced by use of compound No. 63. The sharp return of the prothrombin time to normal after vitamin-$K_1$ administration is quite unlike the gradual return resulting from a spontaneous decrease in anticoagulant effect.

Seven of these 21 patients who received vitamin $K_1$ as an antidote for hypoprothrombinemia induced by compound No. 63 had thromboembolic diseases. No adverse effects were noted in any of these patients as a result of the rapid increase in the prothrombin percentages.

*Vitamin $K_1$ Oxide.*—This preparation was administered, on four occasions to three patients with hypoprothrombinemia induced by compound No. 63, in doses varying from 50 to 150 mg. In all but one instance, maintenance anticoagulant therapy was given simultaneously. The responses were as follows: A prothrombin percentage of 33 increased to 40 and 66% in four and in 20 hr., respectively, with administration of 100 mg. of vitamin $K_1$ oxide in one patient. An additional 100 mg. administered to the same patient produced a change to 84 and 90% in the next four-hour and 20-hr. periods, respectively, without more anticoagulant being given. In another instance, administration of 150 mg. of vitamin $K_1$ oxide increased a value of 33 to 42% in four hours, to 51% in 20 hr., and to 73% in 28 hr., despite maintenance of anticoagulant therapy. Treatment with 50 mg. of this substance caused no alteration in a prothrombin level of 40% in a third patient in four hours

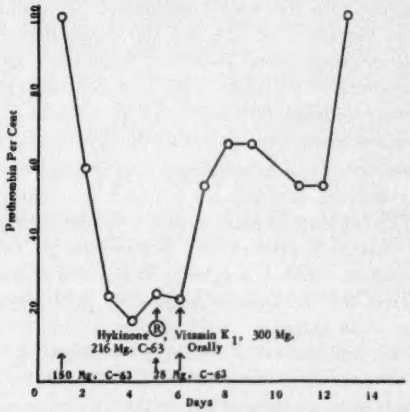


Fig. 5.—A comparison of the effects of vitamin-K (hykinone®) and vitamin $K_1$ administration on hypoprothrombinemia induced and maintained with repeated doses of compound No. 63.

but produced a value of 48% in 20 hr., although 25 mg. of compound No. 63 was administered simultaneously.

*Comparative Effects of Various Antidotes.*—In several instances two or more of the various antidotes were administered to the same patient in an attempt to detect their relative potency in reversing the hypoprothrombinemic effect of compound No. 63. These results are depicted in Figures 3, 4, and 5. In all cases the superiority of vitamin $K_1$ is obvious.

## COMMENT

An ideal anticoagulant has been characterized by Shapiro and Weiner [5] as one which is clinically effective, completely and rapidly absorbed, metabolized uniformly by different subjects, with prompt onset of its therapeutic effect, and devoid of serious toxic effects. So far there is no known drug which possesses all these characteristics. While compound No. 63 does not satisfy all these criteria, it is our contention that this preparation is a better anticoagulant than bishydroxycoumarin.

Anticoagulant No. 63 is capable of producing a therapeutic prothrombin level in 78% of the patients within 48 hr., and, thus, it usually acts more quickly than does bishydroxycoumarin in this respect. This does not obviate the necessity for associated use of heparin when immediate anticoagulant action is required, but it assures one that the prothrombin time will, in most instances, be well within therapeutic limits by the second day.

More important, however, is the fact that in our experience we find that safe and effective prothrombin values are more easily maintained with less fluctuation with this compound than with bishydroxycoumarin. With the exception of one patient, prothrombin determinations were made daily, and until further experience has been accumulated we should advise that this be continued. However, it has been demonstrated that therapeutic prothrombin values can be maintained readily with a relatively constant dose of the drug for prolonged periods.

Toxic manifestations with the use of compound No. 63 have been minimal. Only four patients in the series of 124 had significant hemorrhagic phenomena, and extenuating circumstances were associated with three of these cases. The appearance of erythrocytes in the urine with a prolonged prothrombin time was uncommon with therapy with compound No. 63, as opposed to the 14% incidence of microscopic hematuria associated with bishydroxycoumarin administration.[9]

Unfortunately, the criteria of effectiveness and indications for the use of the various antidotes for anticoagulant-induced hypoprothrombinemia have never been clearly evaluated. This, at least in part, accounts for the conflicting reports on the value of the various vitamin-K preparations in reversing the action of bishydroxycoumarin. In our opinion, mere prolongation of a prothrombin time beyond what is ordinarily considered the therapeutic range does not constitute an immediate indication for the use of an antidote. Such patients should be watched closely for bleeding manifestations, and the urine should be examined daily for the presence of red blood cells. Of course, no more anticoagulant should be ordered until the prothrombin time returns to the safe range. The advantage of compound No. 63 over bishydroxycoumarin becomes more obvious in such circumstances, when it is realized that bleeding manifestations are apparently less frequent with the former than with the latter.

The chief indications for the use of an antidote for an anticoagulant are the earliest signs of gross bleeding or when an emergency operation is necessary while the patient has drug-induced hypoprothrombinemia. In such circumstances the antidote of choice should be one that can be readily administered, is prompt in action, and is capable of bringing the prothrombin to a level at which bleeding would be unlikely. From our experience with compound No. 63, the agent which best meets these requirements is vitamin $K_1$. This substance can be administered by mouth, and some effect can usually be observed as early as four hours later. Within 20 hr. the prothrombin is at a level where bleeding due to hypoprothrombinemia would be most unusual. Our experience with vitamin $K_1$ oxide has been limited, but it is our impression that this product is efficacious, too.

Our results with the water-soluble vitamin-K preparations have been quite variable, even when given in very large doses, and, while some effect may be noted, they are not the drugs of choice for control of excessive hypoprothrombinemia. In

the experience of Cromer and Barker,[13] and Shapiro, Weiner, and Simson,[14] as well as Overman, Sorenson, and Wright,[15] the synthetic water-soluble vitamin-K preparations are very effective in bringing a bishydroxycoumarin-induced prolonged prothrombin time into therapeutic range. On the other hand, James and associates [16] compared the various vitamin-K preparations which could be administered intravenously and found that vitamin $K_1$ oxide was the most effective. Miller and co-workers [17] concluded that vitamin $K_1$ and vitamin $K_1$ oxide provide the only effective means, except blood transfusions, of treating the bleeding emergency of bishydroxycoumarin therapy.

The failure of blood transfusions adequately to reverse the hypoprothrombinemic effect of compound No. 63 was quite enigmatic in view of their apparent effectiveness against bishydroxycoumarin. The reason for this is not clear. Although our series was small, the results were consistent, and even fresh blood was of little value. Even when blood samples were tested as early as one hour, and at hourly intervals thereafter, following a blood transfusion, there was no marked alteration of the prothrombin level. One might postulate that the quantity of prothrombin in a 500 cc.-blood sample was insufficient to counteract the deficiency created by the anticoagulant. However, this would not account for the apparent differences in results on attempts at reversal of the effects of compound No. 63 and of bishydroxycoumarin.

## SUMMARY AND CONCLUSIONS

The 4-hydroxycoumarin anticoagulant No. 63 is a safe and effective preparation for use in treatment of thromboembolic disorders. The advantages of this substance over bishydroxycoumarin (dicumarol®) are the earlier appearance of therapeutic prothrombin levels and the ease of maintenance of these values with a fixed dose of the anticoagulant.

Toxic side effects of compound No. 63 are minimal, and the incidence of microscopic hematuria is less than half that occurring after use of bishydroxycoumarin.

In our experience so far, the best antidotes for the hypoprothrombinemia induced by this anticoagulant are vitamin $K_1$ and, possibly, vitamin $K_1$ oxide. Blood transfusions and administration of water-soluble vitamin-K preparations were far from being efficacious in most instances.

The anticoagulant most recently used was supplied by Abbott Laboratories, North Chicago, Ill. Their experimental number for this drug is BL-5.

13. Cromer, H. E., and Barker, N. W.: The Effect of Large Doses of Menadione Bisulfite (Synthetic Vitamin K) on Excessive Hypoprothrombinemia Induced by Dicumarol, Proc. Staff Meet., Mayo Clin. **19**:217 (May 3) 1944.

14. Shapiro, S.; Weiner, M., and Simson, G.: The Effect of Water-Soluble Preparations of Vitamin K in Dicumarol-Induced Hypoprothrombinemia, New England J. Med. **243**:775 (Nov. 16) 1950.

15. Overman, R. S.; Sorenson, C. W., and Wright, I. S.: Effectiveness of Synthetic Water-Soluble Vitamin K Preparations in Bishydroxycoumarin-Induced Hypoprothrombinemia, J. A. M. A. **145**:393 (Feb. 10) 1951.

16. James, D. F.; Bennett, I. L.; Scheinberg, P., and Butler, J. J.: Clinical Studies on Dicumarol Hypoprothrombinemia and Vitamin K Preparations, Arch. Int. Med. **83**:632 (June) 1949.

17. Miller, R.; Harvey, W. P., and Finch, C. A.: Antagonism of Dicumarol by Vitamin K Preparations, New England J. Med. **242**:211 (Feb. 9) 1950.

# EFFECT OF CORTICOTROPHIN AND CORTISONE ON THE BLOOD IN VARIOUS DISORDERS IN MAN

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SINCE the discovery that corticotrophin (ACTH) and cortisone exert beneficial effects on a wide variety of disease processes which had not been considered previously to be primarily hormonal in nature, a number of patients with disorders of the blood have been treated with these agents. This report describes our own studies in 40 cases. A preliminary report of the observations on 24 of these cases has been published.[1]

It is now well known that lymphopenia and neutrophilia develop in experimental animals [2] and in man [3] after administration of corticotrophin. The reduction in circulating eosinophils which follows administration of corticotrophin has been used as a test for adrenal-cortical insufficiency.[4] Alterations have also been produced

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From the Departments of Medicine and Biochemistry, College of Medicine, University of Utah.

These studies were aided by grants from the United States Public Health Service and the Damon Runyon Memorial Fund for Cancer Research.

1. Wintrobe, M. M.; Cartwright, G. E.; Kuhns, W. J.; Palmer, J. G., and Lahey, M. E.: The Effects of ACTH on the Hematopoietic System, Blood **5**:789, 1950. Wintrobe, M. M.: ACTH and Cortisone in Hemopoietic Disorders, Am. J. Med. **9**:715, 1950.

2. (*a*) White, A., and Dougherty, T. F.: The Effect of Prolonged Stimulation of the Adrenal Cortex and of Adrenalectomy on the Numbers of Circulating Erythrocytes and Lymphocytes, Endocrinology **36**:16, 1945. (*b*) Dougherty, T. F., and White, A.: Influence of Hormones on Lymphoid Tissue Structure and Function: Role of the Pituitary Adrenotrophic Hormone in the Regulation of the Lymphocytes and Other Cellular Elements of the Blood, ibid. **35**:1, 1944.

3. Hills, A. G.; Forsham, P. H., and Finch, C. A.: Changes in the Circulating Leukocytes Induced by the Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) in Man, Blood **3**:755, 1948.

4. Thorn, G. W.; Forsham, P. H.; Prunty, F. T. G., and Hills, A. G.: A Test for Adrenal Cortical Insufficiency: Response to Pituitary Adrenocorticotrophic Hormone, J. A. M. A. **137**:1005 (July 17) 1948.

by corticotrophin and cortisone in various disease processes involving the leucocytes. Thus, improvement after corticotrophin therapy has been seen in a number of cases of acute leukemia, with "complete" temporary remissions in some. Improvement has also been described in chronic lymphocytic leukemia, Hodgkin's disease, and certain other lymph-node disorders.[5] Benefit has been reported in leukemia cutis [5a] and in mycosis fungoides,[5e] but little or no improvement has been observed in chronic myelocytic leukemia after treatment with these hormones.[5d] The leucocytosis associated with infections and the leucopenia occurring in such diseases as disseminated lupus erythematosus may be relieved,[6] at least temporarily. Furthermore, the L. E. cells observed in cases of disseminated lupus [7] may decrease or disappear on treatment.[8]

A number of diseases associated with anemia have been reported to be favorably influenced, at least temporarily, by administration of these hormones, and, in association with the improvement, there has been a decrease in the degree of anemia. The conditions affected include rheumatoid arthritis and rheumatic fever,[9] acute

5. (*a*) Stickney, J. M.; Heck, F. J., and Watkins, C. H.: Cortisone and ACTH in the Management of Leukemia and Lymphoblastoma, Proc. Staff Meet., Mayo Clin. **25**:488, 1950. (*b*) Spies, T. D.; Lopez, G. G.; Milanes, F.; Toca, R. L.; Reboredo, A., and Aramburu, M.: A Note on the Effect of Pituitary Adrenocorticotrophic Hormone (ACTH) and Cortisone in Ameliorating the Symptoms of Leukemia and of Cortisone in Hodgkin's Disease, South. M. J. **43**:497, 1950. (*c*) Pearson, O. H.; Eliel, L. P.; Rawson, R. W.; Dobriner, K., and Rhoads, C. P.: ACTH- and Cortisone-Induced Regression of Lymphoid Tumors in Man, Cancer **2**:943, 1949. (*d*) Pearson, O. H., and Eliel, L. P.: Use of Pituitary Adrenocorticotrophic Hormone (ACTH) and Cortisone in Lymphomas and Leukemias, J. A. M. A. **144**:1349 (Dec. 16) 1950. (*e*) Taylor, S. G., III; Ayer, J. P., and Morris, R. S.: Cortical Steroids in the Treatment of Cancer, ibid. **144**:1058 (Nov. 25) 1950. (*f*) Dameshek, W.; Saunders, R. H., Jr., and Zannos, L.: The Use of ACTH in the Treatment of Acute and Subacute Leukemia: Preliminary Note, Bull. New England M. Center **12**:11, 1950.

6. (*a*) Thorn, G. W.; Forsham, P. H.; Frawley, T. F.; Hill, S. R., Jr.; Roche, M.; Staehlin, D., and Wilson, D. L.: The Clinical Usefulness of ACTH and Cortisone, New England J. Med. **242**:783, 824, and 965, 1950. (*b*) Grace, A. W., and Combes, F. C.: Remission of Disseminated Lupus Erythematosus Induced by Adrenocorticotropin, Proc. Soc. Exper. Biol. & Med. **72**:563, 1949.

7. Hargraves, M. M.; Richmond, H., and Morton, R.: Presentation of 2 Bone Marrow Elements: The "Tart" Cell and the "L. E." Cell, Proc. Staff Meet., Mayo Clin. **23**:25, 1948.

8. Thorn and others.[6a] Flood, F. T., and Limarzi, L. R.: Bone Marrow Studies in Lupus Erythematosus Before and After ACTH and Cortisone Therapy, Proc. Central Soc. Clin. Research **23**:35, 1950.

9. (*a*) Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: The Effects of a Hormone of the Adrenal Cortex (17-Hydroxy-11-Dehydrocorticosterone: Compound E) and of Pituitary Adrenocorticotrophic Hormone on Rheumatoid Arthritis: Preliminary Report, Proc. Staff Meet., Mayo Clin. **24**:181, 1949. (*b*) Hench, P. S.; Slocumb, C. H.; Barnes, A. R.; Smith, H. L.; Polley, H. F., and Kendall, E. C.: The Effects of the Adrenal Cortical Hormone 17-Hydroxy-11-Dehydrocorticosterone (Compound E) on the Acute Phase of Rheumatic Fever: Preliminary Report, ibid. **24**:277, 1949. (*c*) Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Effects of Cortisone Acetate and Pituitary ACTH on Rheumatoid Arthritis, Rheumatic Fever, and Certain Other Conditions, Arch. Int. Med. **85**:545 (April) 1950. (*d*) Elkinton, J. R.; Hunt, A. D.; Godfrey, L.; McCrory, W. W.; Rogerson, A. G., and Stokes, J., Jr.: The Effects of Pituitary Adrenocorticotrophic Hormone (ACTH) Therapy, J. A. M. A. **141**:1273 (Dec. 31) 1949.

glomerulonephritis,[10] acute leukemia,[11] acquired hemolytic anemia,[12] disseminated lupus erythematosus,[9d] and Hodgkin's disease.[5b] In many of these cases reticulocytosis and increased erythropoietic activity of the bone marrow were observed, although red-cell changes were not seen in all instances. It is interesting that a tendency to polycythemia has long been noted in Cushing's syndrome [13] and has been observed in mice given prolonged corticotrophin treatment.[2a]

An increase in the thrombocyte (platelet) count has been noted after administration of corticotrophin or cortisone in cases of acute leukemia in which remissions occurred,[14] as well as in idiopathic thrombocytopenic purpura.[15] No changes in thrombocytes have been noted in normal human subjects [6a] or in animals,[16] although in hypophysectomized rats decreased numbers of thrombocytes and megakaryocytes [17] have been reported after injection of these hormones.

## METHODS

The hematological methods used in this study have been described elsewhere.[18] Hemoglobin concentration was determined by the oxyhemoglobin method, using the Evelyn photoelectric colorimeter. The "dry" method of counting reticulocytes was used, and eosinophils were enumerated by Randolph's method.[18] Plasma iron was measured according to the method of Barkan and Walker [19] and plasma copper by the method of Cartwright and associates.[20] Urine for 17-ketosteroid determinations was collected and refrigerated. At the end of each 24-hr. period it was transported to the laboratory, acidified, and extracted with ether. Estrogenic

10. Farnsworth, E. B.: Acute and Subacute Glomerulonephritis Modified by Adrenocorticotropin, Proc. Soc. Exper. Biol. & Med. **74**:57, 1950.

11. (*a*) Stickney and others.[5a] (*b*) Pearson, O. H.; Eliel, L. P., and Talbot, T. R., Jr.: The Use of ACTH and Cortisone in Neoplastic Disease, Bull. New York Acad. Med. **26**:235, 1950.

12. (*a*) Gardner, F. H.; McElfresh, A. E.; Harris, J. W., and Diamond, L. K.: The Effect of Adrenocorticotrophic Hormone (ACTH) in Idiopathic Acquired Hemolytic Anemia as Related to the Hemolytic Mechanisms, J. Lab. & Clin. Med. **37**:444, 1951. (*b*) Dameshek, W.; Rosenthal, M. C., and Schwartz, L. I.: The Treatment of Acquired Hemolytic Anemia with Adrenocorticotrophic Hormone (ACTH), New England J. Med. **244**:117, 1951. (*c*) Davidson, L. S. P.; Duthie, J. J. R.; Girdwood, R. H., and Sinclair, R. J. G.: Clinical Trials of ACTH in Haemolytic Anemia, Brit. M. J. **1**:657, 1951.

13. Wintrobe, M. M.: Clinical Hematology, ed. 2, Philadelphia, Lea & Febiger, 1946.

14. Dameshek and others.[8f] Pearson and others.[11b]

15. (*a*) Meyers, M. C.; Miller, S., and Bethell, F. H.: Administration of ACTH in Hypersplenic Syndromes, Proc. Central Soc. Clin. Research **23**:73, 1950. (*b*) Robson, H. N., and Duthie, J. J. R.: Capillary Resistance and Adrenocortical Activity, Brit. M. J. **2**:971, 1950.

16. Greer, M. A., and Brown, B. R.: Concerning the Relation Between Pituitary Adrenocorticotropin and the Circulating Blood Platelets, Proc. Soc. Exper. Biol. & Med. **69**:361, 1948.

17. Adams, E.: Observations on the Influence of the Hypophysis and the Adrenal Cortex on Blood Platelet Levels, Blood **4**:936, 1949.

18. Randolph, T. G.: Differentiation and Enumeration of Eosinophils in the Counting Chamber with a Glycol Stain: Valuable Technique in Appraising ACTH Dosage, J. Lab. & Clin. Med. **34**:1696, 1949.

19. Barkan, G., and Walker, B. S.: Determination of Serum Iron and Pseudohemoglobin Iron with O-Phenanthroline, J. Biol. Chem. **135**:37, 1940.

20. Cartwright, G. E.; Jones, P. J., and Wintrobe, M. M.: A Method for the Determination of Copper in Blood Serum, J. Biol. Chem. **160**:593, 1945.

compounds were removed by washing with 2.5 N sodium hydroxide solution, and 17-ketosteroids in the ether residue were measured according to the method of Callow, Callow, and Emmens.[21]

The number of patients and the conditions treated are listed in Table 1. In Tables 2 to 11, inclusive, the average or most representative pretreatment blood values in the various cases are presented, as well as the values which demonstrate the greatest change during treatment. This manner of presentation was adopted because in many studies, such as the total leucocyte count, peaks were reached which were unsustained, whereas in others, such as the total serum protein level, a progressive fall or rise occurred throughout treatment. Thus, an average value would not convey an accurate picture.

The dose of drug given varied in each case; the maximum dose per day and total amount in each course are shown in the tables. Corticotrophin [22] was given in divided doses, usually every six hours. All amounts are expressed in terms of Armour Standard LA-1-A. Cortisone [23] was administered in one or two doses daily, intramuscularly or, in one instance, orally (K. M.,

TABLE 1.—*Diseases Treated*

| Disease | No. of Patients | For Details See |
|---|---|---|
| Acute myeloblastic leukemia | 8 | Tables 2 and 3; Figs. 1 and 3 |
| Acute lymphoblastic leukemia | 3 | Tables 2 and 3; Fig. 2 |
| Terminal lymphosarcoma | 1 | Tables 2 and 4; Fig. 4 |
| Multiple myeloma | 1 | Table 4 |
| Chronic lymphocytic leukemia | 2 | Table 5 |
| Hodgkin's disease | 1 | Table 6 |
| Idiopathic neutropenia | 1 | Text |
| Acquired hemolytic anemia | 4 | Table 7; Figs. 5 and 6 |
| Pernicious anemia | 2 | Fig. 7 |
| Aplastic anemia | 3 | Table 8 |
| Myelophthisic anemia | 2 | Table 9 |
| Disseminated lupus erythematosus | 4 | Table 10; Figs. 6 and 8 |
| Rheumatoid arthritis | 3 | Table 11 |
| Acute rheumatic fever | 1 | Table 11 |
| Idiopathic thrombocytopenic purpura | 1 | Fig. 9 |
| Nonthrombocytopenic purpura | 1 | Text |
| Periarteritis nodosa | 1 | Text |
| Portal cirrhosis | 1 | Text |
| Chronic pyelonephritis, uremia | 1 | Text |
| Anemia and leukopenia, cause undetermined | 1 | Text |

aplastic anemia). When treatment with the drugs was discontinued, in most cases the dose was decreased by 12.5 to 25 mg. every one or two days.

### A. EFFECTS IN SPECIFIC DISEASES

1. *Acute Leukemia and Terminal Lymphosarcoma.*—Ten patients with acute leukemia were treated with corticotrophin. One (C. R.) was treated with cortisone. One patient (J. W.) in an acute terminal phase of lymphosarcoma was treated with corticotrophin. All the patients are now dead. The type of leukemia, drugs used, and duration of remission, if any, are shown in Table 2. In Table 3 details are

21. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing the Grouping $-CH_2CO-$ in Urine Extract as an Indication of Androgen Content, Biochem. J. **32**:1312, 1938.

22. All corticotrophin used was supplied by The Armour Laboratories, Chicago 9.

23. 17-Hydroxy-11-dehydrocorticosterone (cortisone) was furnished by Merck & Company, Inc., Rahway, N. J.

TABLE 2.—*Acute Leukemia—Treatment and Duration*

| Patient; Age, Yr.; Sex | Type of Leukemia | Duration Prior to Hormone Therapy | First Course | | | | Duration of Remission, Mo. | Days off Therapy | Second Course | | | | Total Duration of Disease, Mo. | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Drug | Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | | Drug | Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | |
| P. L.; 25; F | Myeloblastic | 2 mo. | ACTH | 35-140 | 2,835 | 41 | 1 | 7 | ACTH | 70-140 | 1,540 | 14 | 3 | Third course: cortisone, 200 mg./day for 6 days |
| S. N.; 36; M | Myeloblastic | 1½ mo. | ACTH | 100-200 | 2,330 | 16 | .. | .. | ..... | ..... | ..... | .. | 2 | ..... |
| K. H.; 7; F | Myeloblastic | 5 mo. | ACTH | 40-70 | 2,285 | 50 | 1 | 0 | Cortisone | 100 | 500 | 5 | 7 | Previous remission (spontaneous) |
| G. C.; 7; M | Myeloblastic | 3 mo. | ACTH | 35-50 | 1,750 | 50 | 2 | 30 | ACTH | 10-50 | 400 | 16 | 6½ | Previous remission ("Aminopterin") |
| H. M. 11; F | Myeloblastic | 2 mo. | ACTH<br>"Aminopterin" | 40-160<br>0.5 | 2,730<br>4.5 | 34<br>9 | .. | .. | ..... | ..... | ..... | .. | 3 | ..... |
| G. A.; 15; M | Myeloblastic | 5½ mo. | ACTH | 100 | 700 | 7 | .. | .. | ..... | ..... | ..... | .. | 5¾ | ..... |
| P. C.; 55; M | Myeloblastic | 20 days | ACTH<br>"Aminopterin" | 50<br>0.5-1.0 | 200<br>7 | 4<br>9 | .. | .. | ..... | ..... | ..... | .. | 1 | ..... |
| S. T.; 19; M | Myeloblastic | 3 mo. | ACTH | 50-100 | 300 | 4 | .. | .. | ..... | ..... | ..... | .. | 3¼ | ..... |
| D. R.; 5; F | Lymphoblastic | 17 mo. | ACTH | 40 | 1,455 | 34 | 2 | 30 | ..... | ..... | ..... | .. | 19½ | Previous remission ("Aminopterin") |
| D. B.; 2; F | Lymphoblastic | 3 mo. | ACTH | 20-40 | 1,555 | 50 | 1 | 7 | "Aminopterin" | 0.5 | 3.0 | 6 | 6 | ..... |
| C. R.; 2½; F | Lymphoblastic | 2½ mo. | Cortisone | 50 | 600 | 6 | .. | 7 | "Aminopterin" | 0.5 | 2.5 | 5 | 3 | Severe local reaction to cortisone |
| J. W.; 6; F | Terminal lymphosarcoma | 3 yr. | ACTH | 50-80 | 1,280 | 14 | .. | .. | ..... | ..... | ..... | .. | 36½ | Previous treatment with nitrogen mustard, x-ray, and transfusion |

given concerning the clinical and laboratory signs of response in these persons. Figure 1 shows the course of the disease in detail in P. L., while Figure 2 presents the course in D. R., both of whom responded well to therapy. P. L. failed to respond to a second course of corticotrophin therapy and to treatment with cortisone, even though the improvement during the first course of corticotrophin administration

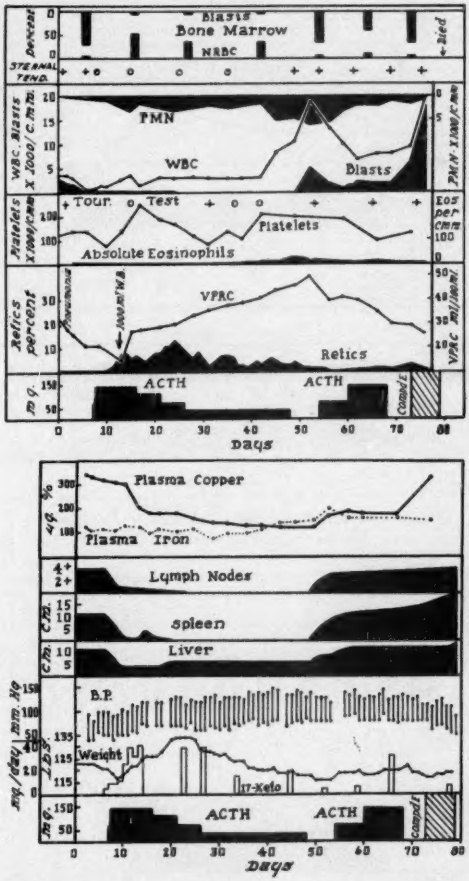


Fig. 1.—Patient P. L., a woman aged 25 with acute leukemia. Note regression in size of liver, spleen, and lymph nodes, as well as decrease in the number of "blast" forms in the peripheral blood and bone marrow and return of normal cellular elements during the first course of corticotrophin (ACTH) therapy. *VPRC* indicates volume of packed red cells.

was so pronounced that all signs of leukemia disappeared and diagnosis at that time would have been impossible. D. R. had had a long remission with "aminopterin" (4-aminopteroyl glutamic acid) therapy. An attack of chickenpox was followed

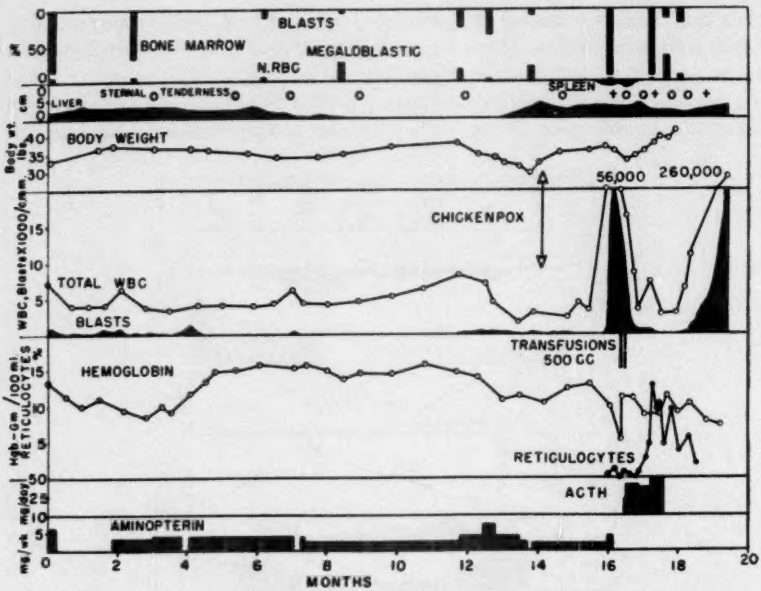


Fig. 2.—Patient D. R., a girl 4 to 5 yr. of age with acute leukemia. Note remissions associated with "aminopterin" therapy, acute infectious disease (chickenpox), and corticotrophin (ACTH) therapy.

TABLE 3.—*Acute Leukemia—Response to Treatment* *

| | | Clinical Signs of Response: Decrease in | | | | | | | Laboratory Signs of Response | | | | | | | Signs of Adrenal Stimulation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Age | Spleen Size | Liver Size | Adenopathy | Fever | Sternal Tenderness | Purpura | Reticulocytosis | Increased V.P.R.C. | Decreased Blasts, P.B.† | Decreased Blasts, B.M.‡ | Total W.B.C. Toward Normal | Increased Thrombocytes | Decreased Bleeding Time or Tourniquet Test | Fall in E.S.R.§ | Decreased Eosinophils | Increased 17-Ketosteroids | Clinical Signs of Hypercorticalism | Comment |
| P. L. | 25 | + | + | + | + | + | + | + | + | + | + | + | — | + | + | 0 | + | + | 1st course, ACTH |
| | | — | — | — | + | — | 0 | — | — | — | + | + | — | — | — | ± | + | 0 | 2d course, ACTH |
| | | — | — | — | — | — | 0 | — | — | — | — | — | 0 | 0 | — | 0 | 0 | 0 | 3d course, cortisone |
| S. N. | 36 | — | — | + | 0 | — | — | — | — | — | — | — | — | — | — | — | + | — | ..... |
| K. H. | 7 | 0 | — | 0 | 0 | — | 0 | + | + | — | + | + | + | + | 0 | 0 | + | + | ..... |
| D. R. | 5 | ± | — | 0 | + | + | 0 | + | — | + | — | + | + | — | 0 | + | + | + | ..... |
| G. C. | 7 | + | + | + | + | + | + | + | — | + | — | + | + | ± | — | + | + | + | 1st course, ACTH |
| | | — | — | — | + | — | — | — | — | + | 0 | + | — | 0 | 0 | + | + | 0 | 2d course, ACTH |
| D. B. | 2 | + | + | ± | + | + | + | + | + | + | + | + | + | + | 0 | ± | 0 | + | ..... |
| H. M. | 11 | — | — | — | — | — | — | — | — | — | — | + | — | — | — | — | ± | — | ..... |
| G. A. | 15 | 0 | 0 | — | ± | 0 | — | — | — | ± | 0 | — | — | 0 | — | — | 0 | — | ..... |
| P. C. | 55 | ± | 0 | — | ± | 0 | 0 | — | — | — | 0 | — | — | 0 | + | — | 0 | — | ..... |
| S. T. | 19 | — | — | — | — | — | — | — | — | — | 0 | — | — | — | + | 0 | 0 | — | ..... |
| C. R. | 2½ | — | — | — | — | — | 0 | — | — | — | 0 | — | — | 0 | 0 | ± | 0 | — | ..... |
| J. W. | 6 | — | ± | — | + | 0 | — | — | — | + | + | — | — | — | — | ± | 0 | — | ..... |

* Plus signs indicate definite response; plus-minus signs, equivocal response; minus signs, no response; zeros, not applicable or not measured.
† Peripheral blood.
‡ Bone marrow.
§ Erythrocyte-sedimentation rate.

by a remission when "aminopterin" treatment no longer appeared to be effective. The subsequent administration of corticotrophin was associated with a brief partial remission.

Figure 3 shows the course of S. N., in whom corticotrophin therapy had no perceptible beneficial effect on the disease. In this patient there was a rapid rise in eosinophils, even though 17-ketosteroid excretion was increased.

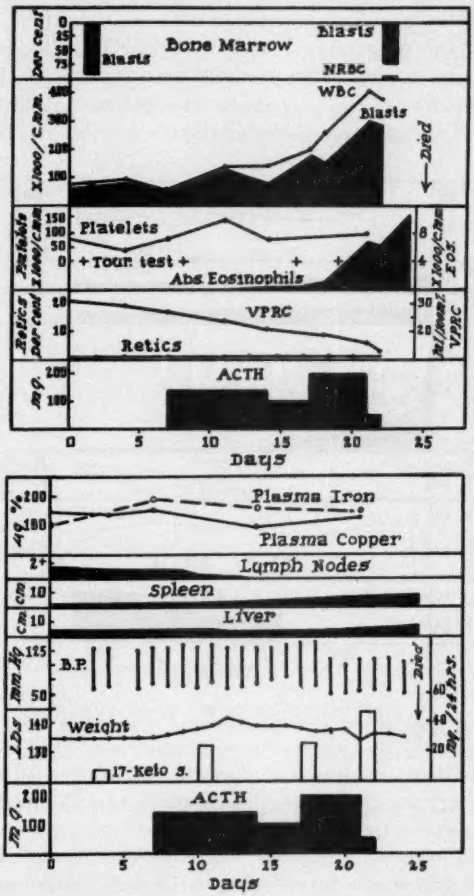


Fig. 3.—Patient S. N., a man aged 36 with acute leukemia. Note lack of clinical or laboratory response, as well as marked increase in eosinophils during corticotrophin (ACTH) therapy.

Of the 12 patients, definite clinical remissions were observed in five (P. L., K. H., D. R., G. C., and D. B.), each of whom was treated with corticotrophin. In only one patient (P. L.) was there a complete remission. In the other four patients remissions were partial and consisted of symptomatic improvement, reduc-

tion in fever, when present, regression of the size of the liver, spleen, and lymph nodes, and reversion toward normal in the abnormalities observed in the blood. In the patient with terminal lymphosarcoma (J. W.) there were a decrease in fever and definite reversion of the bone marrow toward normal shortly before death, but no other change in her condition was noted. The features of her course are presented in Figure 4.

Certain changes took place in seven patients who did not have remissions. These included (1) decrease in fever in three patients (G. A., P. C., J. W.), (2) decrease in adenopathy in one patient (S. N.), (3) decrease in leucocyte count in two (H. M., J. W.), and (4) decrease in sedimentation rate in two (P. C., S. T.).

All five patients who enjoyed remissions relapsed later and died; one (K. H.) relapsed while still receiving corticotrophin therapy and did not respond during a

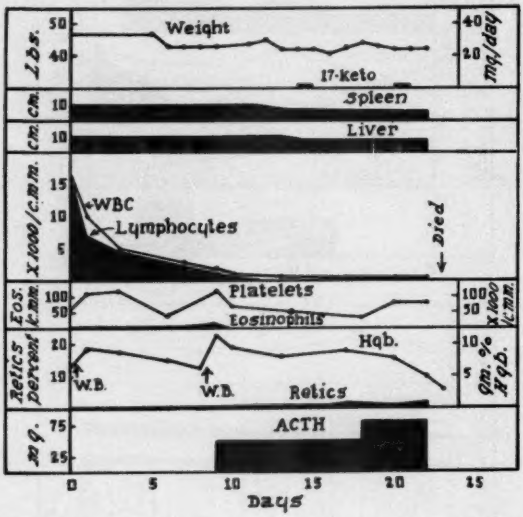


Fig. 4.—Patient J. W., a girl 6 yr. of age with terminal lymphosarcoma. *W. B.* indicates transfusions of whole blood.

five-day course of cortisone treatment. Signs of relapse occurred within one week in four of the five patients, but in one the remission lasted a month. Two patients (P. L., G. C.) were re-treated with corticotrophin, and both showed a decrease in fever and total leucocyte count; the bone marrow of one (P. L.) also showed improvement. In neither was there a significant second clinical remission, however. One of them (P. L.) was then treated with cortisone without effect. In one patient, a child (G. C.), little response occurred during two weeks of corticotrophin therapy at a dose of 40 mg. per day, but when the dose was increased to 50 mg. per day a definite remission took place.

Clinical signs of hypercorticalism were noted in all five patients in whom remissions occurred and in none of the others. Increased 17-ketosteroid excretion was

noted in all five patients with remissions and in one other (S. N.). This measurement was not made for the remainder of the patients with acute leukemia. Eosinophil counts were of little value as a guide to therapy, as in many instances the initial level was quite low.

Two patients (H. M., P. C.) were treated with a combination of "aminopterin" and corticotrophin, but there was no effect other than a decrease in the total number of circulating leucocytes from 18,600 to 3,900 per cubic millimeter in H. M. One patient (C. R.) who showed no response to a short course of treatment with cortisone and one (D. B.) who did respond to corticotrophin administration later showed no response to short courses of "aminopterin" therapy. Three of the patients in whom remissions developed had had previous remissions; two had previously been treated with "aminopterin" and had become refractory to it (D. B., G. C.). In the third patient (K. H.) the previous remission had followed an attack of acute pharyngitis.

Two patients (J. W., P. L.) had pneumonia while receiving therapy. No effect on this disease was noted other than reduction in fever. One patient (P. L.) also

TABLE 4.—*Results of Treatment of Patient with Multiple Myeloma*

| Patient, Age and Sex | Drug | Maximum Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | V.P.R.C., Mil./100 Ml. | Reticulocytes, % | Absolute Number/Cu. Mm. | | | | | Total Protein, Gm./100 Ml. | Globulin, Gm./100 Ml. | E.S.R., Mm./Hr. | Signs of Hypercorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Total W.B.C. | P.M.N. Cells * | Lymphocytes | Plasma Cells | Thrombocytes/Cu. Mm. | | | | | |
| M. W. 56; M | ACTH | 100 | 2,225 | 26 | Before ACTH | 28 | 1.0 | 1,150 | 782 | 230 | 11 | 60,000 | 11.05 | 7.92 | 25 | ..... | ..... |
| | | | | | During therapy | 30 | 6.2 | 5,800 | 4,901 | 841 | 34 | 200,000 | 7.96 | 4.18 | 28 | None | 85.60 |

* Polymorphonuclear cells.

had a breast lesion diagnosed at autopsy as intraductal carcinoma with leukemic infiltration. Drainage from the nipple decreased while she received corticotrophin therapy. A biopsy wound, made just before therapy was commenced, healed satisfactorily. One patient had acute iritis (G. C.), which cleared rapidly while he was receiving corticotrophin.

The duration of life from the time therapy was begun ranged from 55 to 131 days in those who had remissions and from four to 56 days in those who showed no response.

2. *Multiple Myeloma.*—Data on the one patient are presented in Table 4.

In this patient a slight reticulocytosis occurred, but there was no significant change in the volume of packed red cells. The total leucocyte count rose, owing to an increase in neutrophils, and the thrombocytes also increased in number. The most striking change was in the proteins, which fell considerably because of a decrease in globulins. No Bence Jones proteinuria was noted either before or after therapy. Toward the end of the course of treatment the patient displayed suggestive clinical and electrocardiographic signs of hypokalemia. Corticotrophin treatment was discontinued, and potassium therapy was instituted, but the patient, who was in an advanced stage of the disease, died within 48 hr.

3. *Chronic Lymphocytic Leukemia.*—Two patients were treated for periods of about three weeks each. The data are shown in Table 5. In one (H. B.) there was an associated hemolytic anemia, which became less pronounced. In addition, marked euphoria developed and the spleen and liver rapidly decreased in size. He also had persistent diarrhea. The leucocyte count doubled at first but later fell to 32,000 per cubic millimeter while treatment was still being continued. In spite of this, the patient died suddenly, shortly after therapy was stopped. No signs of

TABLE 5.—*Results of Treatment of Patients with Chronic Lymphocytic Leukemia*

| Patient, Age and Sex | Drug | Maximum Dose/ Day, Mg. | Total Amount Given, Mg. | Days Treated | | Spleen, Cm. | Liver, Cm. | Lymph Nodes | V.P.R.C., Ml./100 Ml. | Reticulocytes, % | Absolute Number/Cu. Mm. | | | Signs of Hypereorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Total W.B.C. | P.M.N. Cells | Lymphocytes | | |
| H. B. 74; M | ACTH | 100 | 1,900 | 22 | Before ACTH | 9 | 4 | ± | 19 | 7.8 | 37,750 | 3,788 | 24,108 | .. | 6.75 |
| | | | | | During therapy | 1 | 1 | .. | 30 | 18.6 | 79,500 | 3,900 | 75,525 | + | 36.40 |
| E. G. 49; M | ACTH | 200 | 2,475 | 23 | Before ACTH | 11 | 5 | + | 27 | 0.4 | 53,000 | 2,453 | 42,400 | .. | 5.60 |
| | | | | | During therapy | 6 | 8 | + | 22 | 0.4 | 46,750 | 2,805 | 38,738 | .. | 11.10 |

TABLE 6.—*Results of Treatment of Patient with Hodgkin's Disease*

| Patient, Age and Sex | Drug | Maximum Dose/ Day, Mg. | Total Amount Given, Mg. | Days Treated | | V.P.R.C., Ml./100 Ml. | Reticulocytes, % | Absolute Number/ Cu. Mm. | | | Thrombocytes/ Cu. Mm. | Capillary Fragility | Body Temperature, F | Signs of Hypereorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Total W.B.C. | P.M.N. Cells | Lymphocytes | | | | | |
| J. B. 23; M | ..... | ... | ..... | .. | Before ACTH | 28 | 5.8 | 2,300 | 1,200 | 1,100 | 68,000 | +++ | 103-104 | .. | 2.30 |
| | ACTH (i.m.)* | 100 | 2,050 | 21 | During therapy | 31 | 1.2 | 2,400 | 1,800 | 600 | 58,000 | ++ | 98-99 | + | 9.40 |
| | ACTH (i.v.)† | 12.5 | 188 | 15 | During therapy | 30 | 1.1 | 2,500 | 1,900 | 600 | 78,000 | +++ | 101-103 | .. | 19.00 |

* Intramuscularly.
† Intravenously.

hypokalemia were evident, but it is possible that this may have contributed to his death. In the second patient (E. G.) no change in the disease could be observed, except for moderate reduction in the size of the spleen.

4. *Hodgkin's Disease.*—One patient (J. B.) was treated with corticotrophin. His disease, of approximately one year's duration, had entered a terminal phase at the time of treatment; he showed marked weight loss, fever, anorexia, moderate lymph-node and splenic enlargement, and purpura. Severe pancytopenia and increased capillary fragility were also present. Previous therapy with nitrogen mustard (methyl *bis* or *tris* [2-chloroethyl] amine hydrochloride) and triethylene melamine had had little beneficial effect. Within three days after treatment with

corticotrophin was begun intramuscularly at a dose of 100 mg. per day, his temperature became normal, he became euphoric, and his appetite improved. Questionable reduction in the size of his spleen and in capillary fragility was also noted. These effects continued for two weeks, after which the dose was decreased to 50 mg. per day. On the third day after the dose was decreased, his temperature again rose and symptoms returned. Corticotrophin was then administered intravenously at a rate of 12.5 mg. per 24 hr. No clinical response was obtained, even though his 17-ketosteroid excretion was higher than had been noted during the period of intramuscular corticotrophin administration. Data on this patient are given in Table 6.

5. *Idiopathic Neutropenia.*—A 61-yr.-old man was treated who was subject to recurrent infections and had been known to have a total leucocyte count ranging from 1,000 to 4,000 per cubic millimeter for two years. The leucopenia was due

TABLE 7.—*Results of Treatment of Patients with Acquired Hemolytic Anemia*

| Patient, Age and Sex | Drug | Maximum Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | Spleen, Cm. | V.P.R.C., Ml./100 Ml. | Reticulocytes, % | Serum Bilirubin, Mg./100 Ml. | Coombs Titer | Absolute Number/Cu. Mm. | | | | Signs of Hypereorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Total W.B.C. | P.M.N. Cells | Lymphocytes | Eosinophils | | |
| H. B. 74; M | ACTH | 100 | 1,900 | 22 | Before ACTH | 9 | 19 | 7.8 | 2.6 | 1:2 | 37,750 | 3,788 | 24,106 | 60 | .. | 6.75 |
| | | | | | During therapy | 1 | 30 | 18.6 | 1.0 | 1:2 | 79,500 | 3,900 | 75,525 | 0 | + | 36.40 |
| G. C. 41; M | ACTH | 200 | 2,885 | 20 | Before ACTH | ..* | 15 | 45.4 | 9.5 | 1:4 | 8,150 | 5,542 | 1,793 | 428 | .. | ..... |
| | | | | | During therapy | ..* | 40 | 72.8 | 4.6 | 1:32 | 29,920 | 22,131 | 4,488 | 244 | + | 19.90 |
| L. E. 50; F | ACTH | 100 | 1,980 | 35 | Before ACTH | ..* | 21 | 9.4 | 4.4 | 1:32 | 4,850 | 3,000 | 1,850 | 25 | .. | 2.50 |
| | | | | | During therapy | ..* | 35 | 25.4 | 0.8 | 0 | 9,500 | 7,000 | 2,500 | 6 | + | 11.00 |
| J. M. 70; M | ACTH | 100 | 1,515 | 18 | Before ACTH | 20 | 20 | 10.2 | 0.6 | 0 | 4,200 | 2,856 | 546 | 31 | .. | 2.70 |
| | | | | | During therapy | 13 | 18 | 16.6 | 2.8 | 0 | 9,300 | 6,324 | 1,488 | 28 | .. | 4.80 |

* Splenectomy previously performed.

chiefly to neutropenia. No cause for the neutropenia could be established. Treatment with corticotrophin for 17 days in a dosage of 100 mg. per day resulted in slight euphoria and a rise in urinary 17-ketosteroid excretion from 3.8 to 14.2 mg. per 24 hr. The eosinophil count was 28 per cubic millimeter prior to treatment and ranged from 0 to 6 during treatment. Other than these, however, no effects were noted, either clinically or in the blood smear.

6. *Acquired Hemolytic Anemia.*—Four patients were treated; pertinent data are shown in Table 7.

The course of one patient (G. C.) with acute idiopathic acquired hemolytic anemia is shown in Figure 5. Splenectomy had been performed four days prior to the start of corticotrophin administration, but there had been no improvement in the anemia, and the bilirubinemia and reticulocytosis persisted. Corticotrophin therapy was followed promptly by marked reticulocytosis (72.8%); the serum bilirubin decreased rapidly, and the anemia was relieved. This remission has been

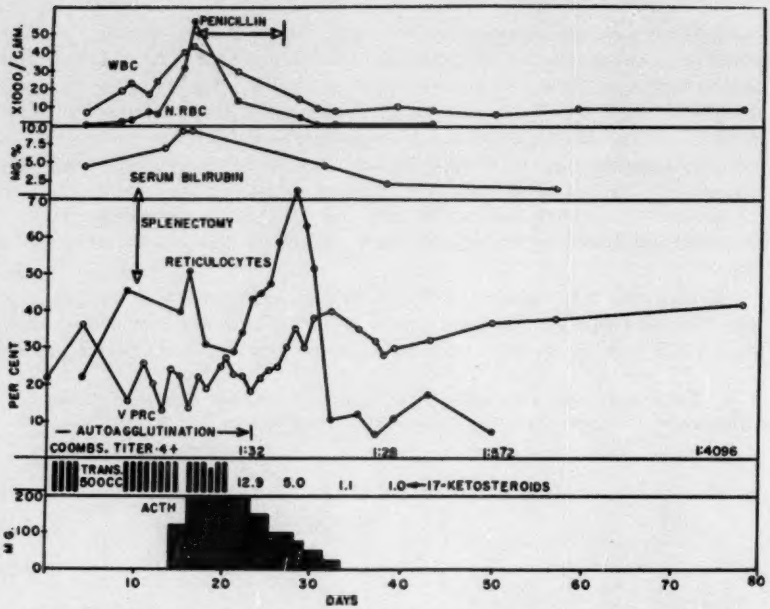


Fig. 5.—Patient G. C., a man aged 41 with idiopathic acquired hemolytic anemia. Note marked reticulocytosis and decrease of anemia associated with rising direct Coombs titer.

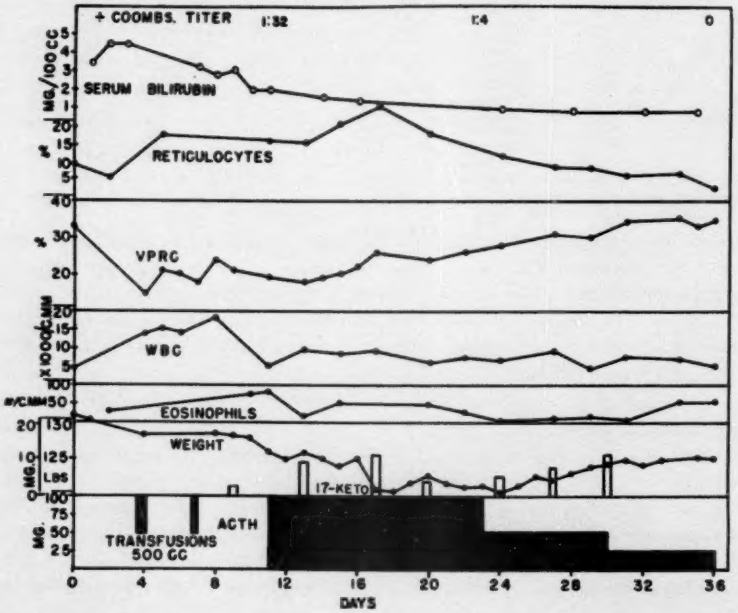


Fig. 6.—Patient L. E., a woman aged 50 with secondary hemolytic anemia in disseminated lupus erythematosus. Marked improvement followed corticotrophin (ACTH) therapy.

maintained for more than nine months. The Coombs titer rose from an initial level of 1 : 32 to 1 : 4,096 at three months and fell to 1 : 32 at eight months.

In two patients the hemolytic process was associated with other diseases. One (H. B.) had chronic lymphocytic leukemia; his response has been discussed already.

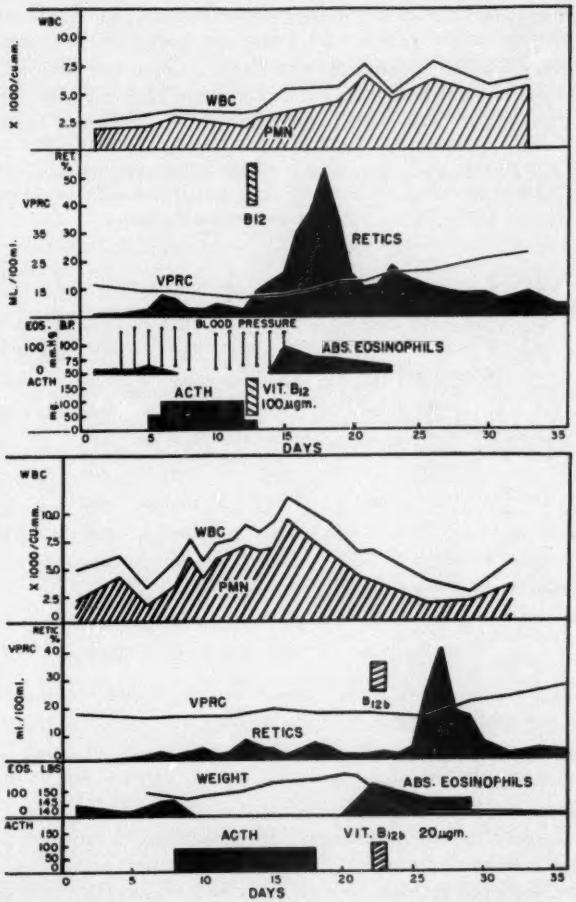


Fig. 7.—Patients R. W., a woman aged 73, and H. N., a man aged 56, with pernicious anemia. Note absence of increase in volume of packed red cells (*VPRC*) and slight reticulocytosis during corticotrophin (ACTH) therapy. A good response to vitamin $B_{12}$ therapy took place in both patients.

During the period of corticotrophin administration, there were an increase in the degree of reticulocytosis, a rise in the volume of packed red cells, and a decrease in serum bilirubin values. The second patient (L. E.) had lupus erythematosus and hemolytic anemia and had been splenectomized two years previously because

of an episode of bleeding with purpura. There was marked symptomatic improvement when corticotrophin was given, the serum bilirubin fell, the volume of packed red cells increased, and the Coombs reaction became negative. The course of this patient is shown in Figure 6.

The fourth patient (J. M.) had chronic hemolytic anemia, leucopenia, a negative Coombs reaction, and a definitely positive reaction to Ham and Horack's test (acid fragility). In spite of the patient's advanced age, paroxysmal nocturnal hemoglobinuria was suspected. Treatment with corticotrophin was followed by relief of the leucopenia and reduction in the size of the spleen, but no change in the hemolytic process was noted. The patient died later. Autopsy revealed marked extra-

TABLE 8.—*Results of Treatment of Patients with Aplastic Anemia*

| Patient, Age and Sex | Drug | Maximum Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | V.P.R.C., Ml./100 Ml. | Reticulocytes, % | Absolute Number/Cu. Mm. | | | | Thrombocytes/Cu. Mm. | Bleeding Time, Min. | Capillary Fragility | Purpura | Signs of Hypercorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Total W.B.C. | P.M.N. Cells | Lymphocytes | Eosinophils | | | | | | |
| P. K. 24; M | ACTH | 100 | 475 | 6 | Before ACTH | 26 | 2.0 | 1,600 | 900 | 891 | .. | 50,000 | 19 | + | ++ | .. | ..... |
| | | | | | During therapy | 28 | 2.8 | 3,150 | 2,362 | 472 | 0 | 70,000 | 31 | + | + | .. | ..... |
| | ACTH | 120 | 1,080 | 10 | Before ACTH | 29 | 2.2 | 1,900 | 600 | 1,100 | 63 | 50,700 | .. | + | + | .. | 10.25 |
| | | | | | During therapy | 31 | 3.4 | 4,250 | 3,400 | 1,020 | 0 | 70,000 | .. | .. | .. | + | 32.50 |
| L. W. 37; M | ACTH | 100 | 1,150 | 13 | Before ACTH | 10 | 2.0 | 2,200 | 625 | 1,300 | 15 | 90,000 | .. | + | + | .. | ..... |
| | | | | | During therapy | 16 | 3.6 | 3,250 | 1,788 | 1,332 | 3 | 90,000 | .. | ± | .. | .. | 24.00 |
| K. M. 29; M | ACTH | 100 | 1,950 | 25 | Before ACTH | 30* | 0.1 | 3,800 | 1,140 | 2,650 | 3 | 50,000 | >20 | + | +++ | .. | ..... |
| | | | | | During therapy | 32* | 0.2 | 5,300 | 2,200 | 2,120 | 12 | 44,000 | >20 | + | +++ | .. | ..... |
| | Cortisone | 200 | 1,300 | 7† | Before cortisone | 20* | 0.6 | 10,300 | 6,950 | 2,540 | 3 | 64,000 | .. | .. | ++ | .. | ..... |
| | | | | | During therapy | 20* | 0.0 | 5,700 | 3,520 | 1,730 | .. | 48,000 | .. | .. | ++ | .. | ..... |

* Frequent transfusions.
† After splenectomy.

medullary myeloid infiltration with little erythropoiesis, and a pathological diagnosis of myelocytic leukemia was made.

*7. Pernicious Anemia.*—Two patients were treated. No symptomatic improvement was observed in either, although a slight increase in reticulocytes without change in the volume of packed red cells or mean corpuscular volume took place. Neutrophilia developed in both. No change could be seen in the bone marrow megaloblasts, the number of thrombocytes, or the neurological findings. Both patients subsequently showed a good response to the administration of vitamin $B_{12}$. Figure 7 depicts the course of these patients.

*8. Aplastic Anemia.*—Three patients with this disease were treated, and two (P. K., L. W.) improved subjectively. No change in physical findings was noted except for diminished purpura in both. No change took place in the volume of packed red cells or reticulocytes. A slight increase in neutrophils occurred in both patients, but no change in thrombocytes or in the bone marrow was noted. In Table

8 it may be seen that capillary fragility, as measured by the tourniquet test, became less pronounced in both patients. The first patient, P. K., was treated twice; the first course was brief and without effect, and no clear improvement followed the second course. Splenectomy was performed after the second course, with reversion toward normal of the pancytopenia and disappearance of purpura. Improvement has been maintained for more than eight months, and he no longer requires transfusions.

The third patient (K. M.) had received a course of treatment with cortisone prior to the time he was seen in this clinic. This had had no perceptible effect and was discontinued. After a period of observation he was treated with corticotrophin. Questionable diminution in purpura was the only effect observed, and, while he was receiving corticotrophin, splenectomy was performed. This was followed by neutrophilic leucocytosis. These changes were of short duration, however, and a subsequent course of cortisone therapy was without effect. The patient died after intracranial hemorrhage. Numerous transfusions had been given throughout the period of observation.

TABLE 9.—*Results of Treatment of Patients with Myelophthisic Anemia*

| Patient, Age and Sex | Drug | Maximum Dose/ Day, Mg. | Total Amount Given, Mg. | Days Treated | | Liver, Cm. | Spleen, Cm. | V.P.B.C., Ml./100 Ml. | Reticulocytes, % | Absolute Number/Cu. Mm. Nucleated R.B.C. | Total W.B.C. | P.M.N. Cells | Lymphocytes | Eosinophils | Thrombocytes/ Cu. Mm. | Marrow | Signs of Hypercorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J. H. 55; M | ACTH | 80 | 1,240 | 20 | Before ACTH | .. | 3 | 26 | 2.8 | 3,900 | 11,600 | 5,983 | 1,500 | 28 | 120,000 | Replaced | .. | 4.2 |
| | | | | | During therapy | .. | 1 | 26 | 20.4 | 17,000 | 24,100 | 17,352 | 1,687 | 31 | 190,000 | Erythroid hyperplasia | .. | 13.5 |
| R. J. 60; F | ACTH | 100 | 1,000 | 11 | Before ACTH | 8 | 22 | 30 | 9.1 | 135 | 5,100 | 2,610 | 1,276 | 44 | 130,000 | Fibrotic | .. | 8.6 |
| | | | | | During therapy | 8 | 22 | 29 | 8.2 | 121 | 7,800 | 5,070 | 1,560 | 50 | 190,000 | ..... | .. | 5.7 |

9. *Myelophthisic Anemia.*—Two patients were treated. The effects of corticotrophin therapy are shown in Table 9. A bone marrow biopsy specimen in the first patient (J. H.) revealed extensive replacement by a malignant process thought to be reticulum-cell sarcoma. In the second (R. J.) myelofibrosis was found. In the first patient definite reticulocytosis, with a definite increase in the number of nucleated red cells in the peripheral blood, occurred during therapy with corticotrophin, and the bone marrow showed a considerable decrease in the number of abnormal cells, with a return of the normal elements. No change was seen in the degree of anemia, but marked neutrophilia was noted, and many immature granulocytes appeared in the blood. This patient, who had shown neurotic traits prior to therapy, became psychotic while being treated and exhibited suicidal tendencies. These subsided when treatment was discontinued. He died eight months later, and autopsy revealed extensive infiltration of the liver, spleen, kidney, and bone marrow with very immature cells thought to be of the myeloid series.

The patient with myelofibrosis (R. J.) showed no change except for a slight transient neutrophilia.

10. *Disseminated Lupus Erythematosus.*—Four patients were treated, one receiving two courses; data on these patients appear in Table 10. In two of the four (E. A. and V. M.), L. E. (lupus erythematosus) cells [7] were readily observed in the marrow but could not be demonstrated after corticotrophin therapy was initiated. Both E. A. and V. M. showed definite remission in all signs and symptoms of the disease, although in both improvement was slow, two to three weeks being required for maximum effect. Hematological changes noted included reticulocytosis, slight rise in volume of packed red cells, and neutrophilic leucocytosis, although in one patient (E. A.) leucopenic levels returned even though therapy with corticotrophin was being continued at the same dose.

The patient who received two courses (V. M.) relapsed promptly after the first course, and the remission which accompanied the second course of treatment was less complete than the first. The course of her disease is shown in Figure 8.

TABLE 10.—*Results of Treatment of Patients with Disseminated Lupus Erythematosus*

| Patient, Age and Sex | Drug | Maximum Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | Symptoms Improved | Liver, Cm. | Spleen, Cm. | V.P.R.C., Ml./100 Ml. | Reticulocytes, % | Absolute Number/Cu. Mm. Total W.B.C. | P.M.N. Cells | Lymphocytes | L.E. Cells in Marrow | Signs of Hypercorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. A. 25; F | ACTH | 100 | 3,960 | 42 | Before ACTH | .. | .. | .. | 25 | 1.0 | 3,550 | 3,089 | 320 | + | .. | 2.50 | ..... |
| | | | | | During therapy | + | .. | .. | 40 | 10.4 | 9,500 | 8,265 | 800 | .. | + | 6.50 | ..... |
| V. M. 21; F | ACTH | 140 | 3,360 | 45 | Before ACTH | .. | 12 | 8 | 29 | 2.0 | 3,450 | 2,131 | 530 | + | .. | 2.30 | Pneumonia |
| | | | | | During therapy | + | 4 | .. | 37 | 10.0 | 14,950 | 13,978 | 673 | .. | + | 47.00 | ..... |
| | ACTH | 80 | 320 | 5 | Before ACTH | .. | .. | .. | 31 | 1.6 | 4,350 | 2,666 | 1,333 | .. | + | 3.10 | Urinary tract infection |
| | | | | | During therapy | + | .. | .. | 36 | 1.8 | 7,150 | 5,577 | 1,359 | .. | + | 5.30 | ..... |
| R. M. 30; F | ACTH | 140 | 2,240 | 23 | Before ACTH | .. | .. | .. | 27 | 0.4 | 3,000 | 2,100 | 308 | .. | .. | 1.80 | Pneumonia |
| | | | | | During therapy | ± | .. | .. | 25 | 16.8 | 10,450 | 7,047 | 243 | .. | .. | 1.60 | ..... |
| L. E. 50; F | ACTH | 100 | 1,980 | 35 | Before ACTH | .. | 4 | .. | 33 | 9.4 | 4,850 | 2,720 | 1,650 | .. | .. | 2.50 | Secondary hemolytic anemia |
| | | | | | During therapy | + | .. | .. | 35 | 23.4 | 9,500 | 7,970 | 950 | .. | + | 11.00 | ..... |

The principal manifestations of lupus in patient L. E., who also had hemolytic anemia and whose course was discussed under that heading, consisted of photosensitive skin lesions, leucopenia, and elevated serum gamma globulin levels. All these became modified in the direction of normal with corticotrophin therapy. She also displayed marked euphoria, and her somewhat dark skin became darker. Improvement has been maintained since corticotrophin therapy was discontinued in this patient; in the two other patients relapses occurred, and one (V. M.) died.

The fourth patient (R. M.) showed no improvement with corticotrophin therapy, the only effects being slight reticulocytosis and neutrophilia, and she also subsequently died.

11. *Rheumatoid Arthritis and Rheumatic Fever.*—Three patients were treated who had rheumatoid arthritis, as well as one patient with rheumatic fever; data

concerning these patients are presented in Table 11. The three who had rheumatoid arthritis showed improvement both objectively and subjectively, but there was prompt relapse when treatment was discontinued. A decreased sedimentation rate and slight neutrophilia were noted during therapy in all three. In one, the volume

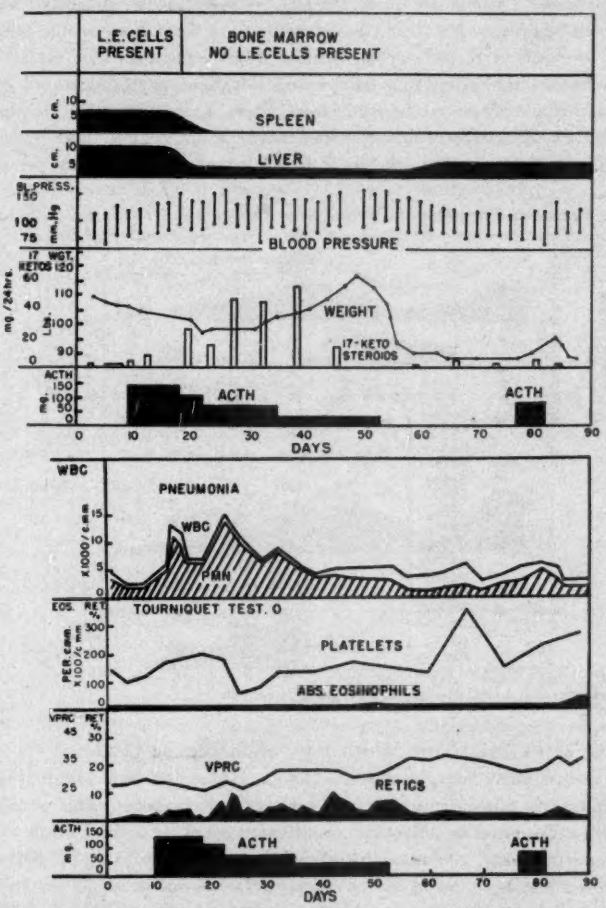


Fig. 8.—Patient V. M., a woman aged 21 with disseminated lupus erythematosus.

of packed red cells rose without reticulocytosis; in the others, no change occurred. Serum copper was studied in two of the three patients. Both showed elevated initial levels, with a reduction during treatment and an increase after treatment was discontinued. No significant change was seen in the lowered serum iron values noted for these patients.

The patient with rheumatic fever was treated with cortisone. Slight subjective improvement without a fall in the sedimentation rate occurred. No change was seen in the blood other than neutrophilia, and the elevated serum copper level did not change.

12. *Idiopathic Thrombocytopenic Purpura.*—One patient was treated who, when seen, showed laboratory but little clinical evidence of thrombocytopenic purpura. A moderate increase in thrombocytes, definite improvement in clot retraction, and definite reduction in bleeding time were noted. Megakaryocytes increased in number in the marrow, and thrombocyte production became more prominent. Within 48 hr. after cessation of corticotrophin administration, the bleeding time became more prolonged. Capillary fragility, the thrombocyte count, and clot retraction remained

TABLE 11.—*Results of Treatment of Patients with Rheumatoid Arthritis and Rheumatic Fever*

| Patient; Age and Sex | Drug | Maximum Dose/Day, Mg. | Total Amount Given, Mg. | Days Treated | | V.P.R.C., Mi./100 Ml. | Reticulocytes, % | Absolute Number per Cu. Mm. | | | | Plasma Iron, γ/100 Ml. | Plasma Copper, γ/100 Ml. | E.P.,* γ/100 Ml. R.B.C. | Signs of Hypercorticalism | Urinary 17-Ketosteroids, Mg./24 Hr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Total W.B.C. | P.M.N. Cells | Lymphocytes | Eosinophils | | | | | |
| I. M. 33; F | ACTH | 100 | 1,225 | 15 | Before ACTH | 33 | 3.2 | 11,750 | 7,637 | 2,703 | 118 | 87 | 186 | ... | .. | 3.5 |
| | | | | | During therapy | 39 | 3.0 | 19,050 | 14,283 | 2,858 | 27 | 43 | 108 | ... | + | 27.3 |
| C. S. 30; F | ACTH | 70 | 1,190 | 17 | Before ACTH | 31 | 1.4 | 7,650 | 5,508 | 1,760 | 156 | 31 | 162 | ... | .. | 4.0 |
| | | | | | During therapy | 27 | 3.6 | 9,400 | 7,463 | 1,890 | 42 | 31 | 114 | 224 | + | 26.0 |
| J. F. 25; M | ACTH | 40 | 400 | 10 | Before ACTH | 34 | 0.4 | 6,750 | 4,658 | 1,687 | 200 | 26 | ... | 194 | .. | .... |
| | | | | | During therapy | 33 | 2.6 | 8,700 | 6,525 | 1,814 | 234 | 25 | ... | ... | .. | .... |
| R. B.† 27; F | Cortisone | 100 | 1,195 | 16 | Before cortisone | 39 | 1.6 | 8,200 | 4,980 | 2,573 | 216 | .. | 216 | ... | .. | .... |
| | | | | | During therapy | 38 | 1.4 | 18,100 | 13,937 | 1,742 | 181 | .. | 192 | ... | .. | .... |

* Free erythrocyte protoporphyrin, micrograms per 100 ml. of red cells.
† Acute rheumatic fever.

normal for seven days, after which they again became abnormal. A complete response was obtained later, after splenectomy. Her course is shown in Figure 9.

In addition to this patient, one with severe nonthrombocytopenic purpura, associated with acute renal insufficiency, was treated for three days, during which time both the purpura and the renal insufficiency rapidly improved. However, slight improvement had been noted in the purpura for 24 hr. prior to the initiation of corticotrophin therapy. Consequently, the results cannot be attributed with certainty to the drug. This patient exhibited a psychosis on the third day of therapy which cleared approximately one week later, after corticotrophin treatment had been discontinued.

13. *Miscellaneous Disorders.*—One child with periarteritis nodosa demonstrated on a muscle biopsy specimen was treated with corticotrophin. No effect was noted on the clinical or laboratory signs of the disease; the patient's condition became progressively worse, and he died after 15 days of therapy.

One patient with portal cirrhosis and one with anemia associated with uremia and chronic pyelonephritis received corticotrophin therapy. No effects on the blood, other than neutrophilia and a slight decrease in the sedimentation rate, were noted.

One patient with severe chronic anemia and leucopenia, for which no causation could be established, was treated with corticotrophin. Again, no effects were observed.

B. GENERAL EFFECTS

1. *Signs of Hypercorticalism.*—The most frequent evidence of adrenal stimulation by corticotrophin was an increase in urinary 17-ketosteroids. This determination was made during 30 courses of therapy in 26 patients. An increase in 17-ketosteroid excretion was noted during 26 of the 30 courses, although it was of slight degree in six. Initially the levels in many of the patients were abnormally

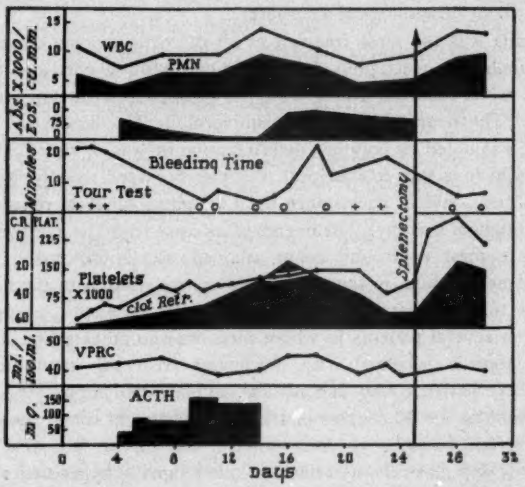


Fig. 9.—Patient N. S., a woman aged 27 with idiopathic thrombocytopenic purpura. Note decrease in bleeding time and capillary fragility (*tour. test*), improved clot retraction, and rise in thrombocyte (platelet) count associated with corticotrophin (ACTH) therapy.

low. Objective signs of alteration in the disease were noted in three patients in whom no rise in 17-ketosteroid excretion was found; conversely, an increase in 17-ketosteroid excretion occurred in nine patients in whom no signs of remission were noted and in three in whom no other evidence of hypercorticalism was observed.

Rounding of the face was the next most frequent sign of hypercorticalism seen, and this occurred in 17 patients. Only four patients with this sign presented no objective evidence of remission; these included one patient each with pernicious anemia (H. N.), aplastic anemia (L. W.), disseminated lupus erythematosus (R. M.), and portal cirrhosis (F. M.). It is noteworthy that all the patients with acute leukemia in whom remissions were observed displayed rounded facial contours, whereas none of the others did.

Other signs of hypercorticalism were seen much less frequently and in all cases were associated with rounded faces. Increase in pigmentation occurred in four

patients, acne in four, and hirsutism of the face in two. Striae developed in one patient, and one child with acute leukemia (G. C.) acquired a pronounced interscapular fat pad. Significant hypertension developed in eight patients, and five patients showed significant weight gain and edema. Glycosuria and hyperglycemia were not looked for routinely but were observed incidentally in three patients.

Euphoria and increased appetite were noted in most, but not all, of the patients in whom symptoms were severe prior to therapy. Psychoses developed in two patients, both of whom had shown psychoneurotic traits prior to therapy. In the majority of the patients who were febrile, a reduction in fever followed initiation of therapy within 24 hr.

2. *Effects on the Blood.*—(*a*) Effects on the White Cells: The known effects of corticotrophin administration on the leucocytes (neutrophilia, lymphopenia, and eosinopenia) were noted with less frequency in these patients than has been reported in patients without hematological disorders.[8]

Neutrophilia was the most frequent effect of corticotrophin administration. A rise in the number of neutrophils during corticotrophin or cortisone therapy above pretreatment levels occurred during 34 of 46 courses of treatment, that is, in 74% of the cases. The neutrophilia was unequivocal in 23 courses, or 50%. In the remainder, it was noted on only one determination or was of very slight magnitude. In those in which it was unequivocal, the rise occurred usually within the first week of treatment, and in no instance did the neutrophil level remain consistently increased throughout therapy. The degree of increase from the average pretreatment count to the highest neutrophil count attained was approximately 100%. The occurrence of neutrophilia in these patients bore no relation to the development of a remission or to the appearance of any of the manifestaitons of Cushing's syndrome; it took place in several patients in whom there was no other effect of therapy.

Lymphocytopenia occurred with much less frequency than other white-cell changes in these patients. An unequivocal decrease in lymphocytes was observed during only nine of the 46 courses of treatment. In eight other cases an equivocal decrease occurred. Lymphocytopenia, when it did appear, was also irregular and unsustained. It was more closely related to other signs of hypercorticalism than was the neutrophilia. In eight of the nine courses in which unequivocal lymphocytopenia was observed, either a definite clinical response or definite signs of hypercorticalism appeared.

In 10 of the 46 courses of treatment the level of eosinophils was too low to allow one to draw any conclusions concerning the effects of treatment. Of the remaining 36, in 11 courses, or 31%, unequivocal eosinopenia developed. However, in 25 instances, or 69%, a tendency toward eosinopenia was noted during therapy. In those cases in which there was unequivocal eosinopenia, the drop was greater than 50% and the count remained low during the course of treatment, the number of eosinophils rising promptly when treatment was discontinued. Only one patient with definite eosinopenia failed to show other evidences of cortical stimulation; in this patient urinary 17-ketosteroids were not measured. Three patients without eosinopenia, however, showed definite signs of hypercorticalism, one exhibiting a wide facial contour and the other two a marked rise in urinary 17-ketosteroids. Indeed, in two of these, the eosinophils rose significantly during treatment.

There was an over-all tendency for the total leucocyte count to rise, but this was inconsistent. No consistent changes in monocytes or basophilic leucocytes were noted.

(*b*) Effects on Red Cells and Thrombocytes: When complete or partial remission of the disease being treated took place, anemia, when present, tended to decrease and the thrombocyte count, if low, rose. These changes were sometimes accompanied with reticulocytosis. Thus, the volume of packed red cells increased in eight patients, in each of whom the rise was associated with a remission in the disease. Three of these patients had acute leukemia; two had acquired hemolytic anemia; two had disseminated lupus erythematosus, and one had anemia associated with rheumatoid arthritis. An increase in thrombocytes was noted in six patients; four had acute leukemia; one had idiopathic thrombocytopenic purpura, and one had multiple myeloma.

Slight reticulocytosis was observed in the two patients with pernicious anemia, in one with myelophthisic anemia, and in one with multiple myeloma. An increase in the number of circulating nucleated red blood cells took place in one of the patients with myelophthisic anemia (J. H.). These changes, however, were not accompanied with evidence of any benefit from the hormone administration.

(*c*) Effects on Serum Iron and Copper: No consistent effects on serum iron were noted in the 15 patients for whom this measurement was made. In four the serum iron was initially low and remained so, even though there was a rise in the volume of packed red cells in two of them.

Serum copper was measured in 15 patients, and in four an abnormally high level was present initially. A significant drop occurred in four patients, including one with an abnormally high initial level. These patients included two with acute leukemia and two with rheumatoid arthritis.

## COMMENT

Among those diseases which we have treated, corticotrophin or cortisone appears to have produced significant changes in the pathological process, with accompanying improvement in the clinical condition of the patient, in certain cases of acute leukemia, acquired hemolytic anemia, disseminated lupus erythematosus, rheumatoid arthritis, and idiopathic thrombocytopenic purpura. The mechanism whereby these substances affect such a variety of apparently unrelated diseases is obscure. An explanation might be sought through an examination of their effects on the hemopoietic system and on immune mechanisms.

Neutrophilia was the most frequently observed change in our cases. It developed in many patients in whom no other effects of treatment were noted. The fact that in our study the appearance of neutrophilia bore no relation to the development of other evidences of adrenal-cortical stimulation is consistent with the observations that neutrophilia occurs after the administration of corticotrophin to adrenalectomized animals [2b] and to patients with Addison's disease.[8] Thus, neutrophilia following corticotrophin administration apparently does not require the presence of an intact adrenal cortex. Data from this laboratory, reported elsewhere,[24] confirm

24. Palmer, J. G.; Cartwright, G. E., and Wintrobe, M. M.: The Effect of ACTH and Cortisone on the Blood of Experimental Animals, in Proceedings of the Second Clinical ACTH Conference, Vol. I: Research, edited by J. R. Mote, Philadelphia, The Blakiston Company, 1951, p. 438.

this finding and indicate that cortisone does not produce neutrophilia in animals. However, one of our patients (R. B.) and patients reported on by others [25] did show striking neutrophilia after the administration of cortisone. It would appear, then, that neutrophilia, which may be produced by corticotrophin in the absence of the adrenal gland, may also be produced in man by adrenal-cortical hormone. At any rate, it does not seem likely that an increase in the number of circulating neutrophils played an important part in the production of remissions in the diseases studied.

When eosinopenia occurred in our patients it was, with the exception of one case, associated with other changes attributable to the hormone administration. In a number of patients, on the other hand, no pronounced eosinopenia followed the administration of these agents. This, however, was of no significance, since many of the patients had very low initial eosinophil counts. Of interest, nevertheless, is the fact that in others there were increased numbers of eosinophils even though signs of hypercorticalism were present. Thus, it is apparent that other factors than cortical hormones influence the number of eosinophils.

Dissolution of lymphatic tissue, with a reduction in the number of circulating lymphocytes, has been known to occur after the administration of corticotrophin or adrenal-cortical hormones.[2] It was these observations which led to studies in animals concerning the relation between the adrenal glands and leukemia and lymphatic tumors and to the trial of these hormonal substances in patients.[26] Heilman and Kendall [27] reported the arrest of growth in malignant lymphoid tumors in the mouse treated with cortisone. Murphy and Sturm [28] showed that adrenalectomy increased the susceptibility of rats to transplanted leukemia and lymphosarcoma and that adrenal implants or administration of adrenal-cortical hormones or of corticotrophin decreased susceptibility. It has not been established, however, that the lymphocytolytic and the antineoplastic effects of the adrenal cortex are related.

Lymphopenia was seen infrequently in our patients. One reason for this undoubtedly is that in many instances studies were not made at the ideal time intervals. In many, furthermore, the nature of the disease studied would have masked the appearance of lymphocyte changes. When it did occur, lymphopenia was closely correlated with other changes indicative of adrenal-cortical hyperfunction.

Polycythemia has been reported in mice treated with corticotrophin [24] and is known to be a feature of Cushing's syndrome.[13] Polycythemia was not observed in any of our patients, however, even in those in whom no anemia was present initially, nor have we found it in rats treated for prolonged periods.[24]

25. (*a*) Perera, G. A.; Fleming, T. C., and Pines, K. L.: Cortisone in Hypertensive Vascular Disease, J. Clin. Invest. **29**:730, 1950. (*b*) Schrock, C. E.; Sheets, R. F., and Bean, W. B.: Observations During ACTH and Cortisone Administration to a Patient with Long Standing Panhypopituitarism and Rheumatoid Arthritis, ibid. **30**:174, 1951. (*c*) Irons, E. N.; Ayer, J. P.; Brown, R. G., and Armstrong, S. H.: ACTH and Cortisone in Diffuse Collagen Disease and Chronic Dermatoses, J. A. M. A. **145**:861 (March 24) 1951.

26. Dougherty and White.[2b] Footnote 5*c* and *f*.

27. Heilman, D. H., and Kendall, E. C.: Influence of 11-Dehydro-17-Hydroxy Corticosterone (Compound E) on Growth of Malignant Tumor in the Mouse, J. Endocrinol. **34**:416, 1944.

28. Murphy, J. B., and Sturm, E.: The Effect of Cortical and Pituitary Adrenotropic Hormones on Transplanted Leukemia in Rats, Science **99**:303, 1944. Sturm, E., and Murphy, J. B.: The Effect of Adrenalectomy on the Susceptibility of Rats to Transplantable Leukemia, Cancer Res. **4**:384, 1944. Murphy, J. B., and Sturm, E.: The Effect of Adrenal Grafting on Transplanted Lymphatic Leukemia in Rats, ibid. **10**:191, 1950.

Hypoferremia and hypercupremia, which have been reported in patients with anemia associated with chronic infections,[29] were also observed in the patients with rheumatoid arthritis. A relation between hypoferremia and the adrenal cortex has been suggested.[30] It is of interest that these changes were not regularly reversed by hormone treatment, even when improvement in the anemia occurred. Hypoferremia was not altered significantly in any patient; the serum copper values were observed to fall in four instances, although there was no correlation between this decrease and the presence of high initial levels.

There is considerable evidence to suggest a relation between the adrenal cortex and immune mechanisms. Dougherty, Chase, and White [31] noted increases in circulating-antibody titers following stimulation of the adrenal cortex or administration of adrenal-cortical hormones. Although others [32] have been unable to produce this effect, there is evidence that the administration of corticotrophin or cortisone influences the response to antigens, in that it inhibits the development of the Arthus phenomenon [33] and interferes with certain skin reactions, such as the tuberculin reaction.[34] This subject has been reviewed by Kass and Finland.[35]

In addition to the above known effects, it has been suggested [6f] that corticotrophin and cortisone may represent specific hemopoietic stimulants. The polycythemia and neutrophilia already mentioned have been cited as manifestations of this effect. The increase in thrombocytes in the patient with idiopathic thrombocytopenic purpura might be another example. White and Dougherty [28] reported increased extramedullary hemopoiesis in animals. In our patients the principal alterations

29. Cartwright, G. E.; Lauritsen, M. A.; Jones, P. J.; Merrill, I. M., and Wintrobe, M. M.: The Anemia of Infection: I. Hypoferremia, Hypercupremia, and Alterations in Porphyrin Metabolism in Patients, J. Clin. Invest. **25**:65, 1946.

30. Cartwright, G. E.; Hamilton, L. D.; Gubler, C. J.; Fellows, N. M.; Ashenbrucker, H., and Wintrobe, M. M.: The Anemia of Infection: XIII. Studies in Experimentally Produced Acute Hypoferremia in Dogs and the Relationship of the Adrenal Cortex to Hypoferremia, J. Clin. Invest. **30**:161, 1951.

31. Dougherty, T. F.; White, A., and Chase, J. H.: Relationship of the Effects of Adrenal Cortical Secretion on Lymphoid Tissue and Antibody Titre, Proc. Soc. Exper. Biol. & Med. **56**:28, 1944. Dougherty, T. F.; Chase, J. H., and White, A.: Pituitary-Adrenal Cortical Control of Antibody Release from Lymphocytes: Explanation of the Anamnestic Response, ibid. **58**:135, 1944. Chase, J. H.; White, A., and Dougherty, T. F.: Enhancement of Circulating Antibody Concentration by Adrenal Cortical Hormones, J. Immunol. **52**:101, 1946.

32. Eisen, H. N.; Mayer, M. M.; Moore, D. H.; Tarr, R., and Stoerk, H. C.: Failure of Adrenal Cortical Activity to Influence Circulating Antibodies and Gamma Globulin, Proc. Soc. Exper. Biol. & Med. **65**:301, 1947. Craddock, C. G., Jr.; Valentine, W. N., and Lawrence, J. S.: The Lymphocyte, J. Lab. & Clin. Med. **34**:158, 1949. De Vries, J. A.: The Effect of Adrenocorticotrophic Hormone on Circulating Antibody Levels, J. Immunol. **65**:1, 1950. Herbert, P. H., and De Vries, J. A.: The Administration of Adrenocorticotrophic Hormone to Normal Human Subjects: Effect on the Leukocytes in the Blood and on Circulating Antibody Levels, Endocrinology **44**:259, 1949.

33. Germuth, F. G., Jr., and Hinnger, B. O.: Effect of 17-Hydroxy-11-Dehydrocorticosterone (Compound E) and of ACTH on Arthus Reaction and Antibody Formation in the Rabbit, Proc. Soc. Exper. Biol. & Med. **74**:815, 1950.

34. Stoerk, H. C.: Inhibition of the Tuberculin Reaction by Cortisone in Vaccinated Guinea Pigs, Federation Proc. **9**:345, 1950.

35. Kass, E. H., and Finland, M.: The Role of Adrenal Steroids in Infection and Immunity, New England J. Med. **244**:464, 1951.

in the blood seen in the diseases other than leukemia were those of increased bone marrow activity. In the patients with hemolytic anemia, greater percentages of reticulocytes were seen, and slight reticulocytosis developed even in the patients with pernicious anemia, disseminated lupus erythematosus, multiple myeloma, and myelophthisic anemia, although in many instances there was no subsequent alteration in the volume of packed red cells. Erythroid hyperplasia in the marrow was seen to develop in a patient with terminal lymphosarcoma and in one with myelophthisic anemia. In the latter patient, a marked increase in nucleated red cells in the peripheral blood was noted in the absence of alteration in the anemia. Other authors [35e] have observed bone marrow stimulation following corticotrophin and cortisone therapy, although, using a quantitative method for determining cell concentration, Yoffey and his co-workers [36] could find no significant change. Obviously these hormones do not stimulate hemopoiesis under all conditions, and it would seem more likely that, in many cases, instead of directly stimulating the bone marrow, they act in some way to alter the process which has made the marrow function abnormally. In view of the alterations in immune mechanisms which have been reported to follow adrenal stimulation, and since acquired hemolytic anemia is frequently accompanied with evidence of the presence of abnormal antibodies, the trial of corticotrophin and cortisone in treatment of this disorder might be expected to be successful. Beneficial effects have been reported by Gardner,[12a] Dameshek,[12b] and Davidson.[12c] It is noteworthy that of the four patients treated in our own study the only one who showed no response (J. M.) also had no demonstrable abnormal antibodies. One patient (G. C.) curiously showed a steady increase in the Coombs titer, even though the rate of hemolysis decreased. The prolonged remissions in these patients are encouraging and suggest that prolonged or even permanent remission may possibly be produced by administration of corticotrophin or cortisone, or that at least the patient may be splenectomized with more safety and perhaps with greater likelihood of success.

It has been suggested that the pathogensis of idiopathic thrombocytopenic purpura is similar to that postulated for acquired hemolytic anemia; namely, that it is the result of the development of abnormal antibodies which produce destruction of thrombocytes.[37] If this is true, the response of idiopathic thrombocytopenic purpura to therapy with corticotrophin and cortisone is not surprising. The patient reported on here apparently had a brief remission; larger series have been reported on by Meyers and co-workers [15a] and by Robson and Duthie,[15b] and in a number of their patients prolonged remissions were observed. Even if remissions are of short duration, however, corticotrophin or cortisone therapy might be useful in decreasing the bleeding tendency so that the operative procedure would be made easier and the risk of serious bleeding prior to operation might be lessened.

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36. Yoffey, J. M.; Metcalf, W. K.; Herdan, G., and Nairn, V.: Effect of ACTH and Suprarenal Extract on Bone Marrow, Brit. M. J. **1**:660, 1951.

37. Evans, R. S., and Duane, R. T.: Acquired Hemolytic Anemia: I. Relation of Erythrocyte Antibody Production to Activity of the Disease; II. Significance of Thrombocytopenia and Leukopenia, Blood **4**:1196, 1949. Evans, R. S.; Takahashi, K.; Duane, R. T.; Payne, R., and Liu, C. K.: Primary Thrombocytopenic Purpura and Acquired Hemolytic Anemia, A. M. A. Arch. Int. Med. **87**:48 (Jan.) 1951.

The results on study of purpura haemorrhagica do not support the thesis of Wiseman and colleagues [38] that this disease is due to increased phagocytosis of thrombocytes by the reticuloendothelial system, since it has been shown [39] that increased adrenal-cortical activity augments the activity of the reticuloendothelial system, at least in certain respects.

Of the patients with acute leukemia treated in this study, the only ones with remissions were those in whom definite manifestations of adrenal-cortical stimulation were noted. This suggests that the dose and duration of treatment should depend on the response in each patient. The dose should be great enough and treatment of long enough duration to produce either a clinical remission or signs of adrenal-cortical stimulation, such as changes in facial contour or increased urinary 17-ketosteroid excretion.

With a comparatively small number of cases of acute leukemia, it is difficult to draw conclusions concerning the factors which influence the occurrence of remissions. The type of leukemia was apparently not important. Eight patients were considered to have myeloblastic leukemia and three lymphoblastic. Remissions occurred in three patients with myeloblastic leukemia and in two with the lymphoblastic form. Age was also not a deciding factor. Eight children and four adults were treated. Remissions occurred in four children and one adult. The duration of the disease prior to treatment ranged from two to 17 mo. in those who had remissions and from 20 days to five and a half months in those who did not. The severity of the disease and the level of the initial leucocyte count showed no consistent relation to the occurrence of remission.

The duration of corticotrophin- and cortisone-induced remissions in our patients with acute leukemia was less than the duration of "aminopterin"-induced remissions observed by others [40] as well as by ourselves. The frequency of remissions has been about the same after treatment with either of these agents. In 29 patients with acute leukemia treated with "aminopterin," we observed remissions of varying degrees in 10. The duration of remissions varied from two to 64 weeks, with an average of 15.2 weeks.

In the two patients for whom a combination of corticotrophin and "aminopterin" therapy was used, no remissions were obtained. However, animal studies [24] have shown that corticotrophin augments the toxic effects of "aminopterin," a result which suggests that a further trial of this combination might be of interest.

The other two diseases in which remissions were noted in this study, namely, disseminated lupus erythematosus and rheumatoid arthritis, have been found to

38. Wiseman, B. K.; Doan, C. A., and Wilson, S. J.: The Present Status of Thrombocytopenic Purpura with Special Reference to Diagnosis and Treatment, J. A. M. A. **115**:8 (July 6) 1940.

39. Gordon, A. S., and Katsch, G. F.: Adrenals and Phagocytosis, Ann. New York Acad. Sc. **52**:1, 1949.

40. Dameshek, W.; Freedman, M. H., and Steinberg, L.: Folic Acid Antagonists in the Treatment of Acute and Subacute Leukemia, Blood **5**:898, 1950. Farber, S.; Downing, V.; Schwachman, H.; Toch, R.; Appleton, R.; Heald, F.; King, J. P., and Feriozi, D.: The Action of ACTH and Cortisone in Acute Leukemia, in Proceedings of the Second Clinical ACTH Conference, Vol. II: Therapeutics, edited by J. R. Mote, Philadelphia, The Blakiston Company, 1951, p. 251.

relapse promptly when therapy is discontinued.[41] Therefore, it seems that the aim of treatment in these disorders should be to find methods for long-term maintenance with these hormones.

Alterations in electrolyte and water metabolism were the most dangerous complications of therapy in these patients. During treatment it is necessary to be on the watch for changes in blood pressure and in weight, and signs of hypokalemia must be looked for. Failure of wound healing [42] and impairment in recovery from infections [35] have been noted in patients and animals. No such ill effect was seen in our patient (P. L.), in whom a biopsy was performed prior to corticotrophin therapy. Four patients (P. L., J. W., K. M., and R. M.) had pneumonia while receiving therapy, and no change in the course of the disease was noted. Care seems indicated, however, in the treatment of patients with these complications.

## SUMMARY

Corticotrophin (ACTH) or cortisone was administered to 40 patients with a variety of disorders affecting the hemopoietic system. Striking improvement in the clinical condition, as well as in the hematological picture, was noted in five of 11 patients with acute leukemia, in three of four patients with acquired hemolytic anemia, in two of four patients with disseminated lupus erythematosus, in all three patients with rheumatoid arthritis, and in one patient with idiopathic thrombocytopenic purpura. In addition, one patient each with multiple myeloma, terminal lymphosarcoma, chronic lymphocytic leukemia, and reticulum-cell sarcoma involving the bone marrow showed a diminution in the number of abnormal cells in the marrow and regeneration of erythroid cells, although there was no clinical improvement. The last patient, who had myelophthisic anemia, showed a marked increase in the number of nucleated red cells in the peripheral blood. One patient with advanced Hodgkin's disease displayed symptomatic improvement and reduction in fever but no hematological change. Slight reticulocytosis with no other effects was noted in two patients with pernicious anemia in relapse, and there was a decrease of purpura in two patients with aplastic anemia. No definite effects, other than the appearance of manifestations of adrenal-cortical hyperfunction, were noted in patients with chronic lymphocytic leukemia, periarteritis nodosa, myelofibrosis, anemia associated with chronic pyelonephritis, portal cirrhosis, and chronic idiopathic neutropenia.

The mode of action of these hormones in the disorders studied is unknown, but it may be related to the known effects of adrenal-cortical hormones in producing lymphocytolysis, increased hemopoiesis, and alterations in immune reactions.

Therapy with these hormones may be of considerable value in the management of patients with acquired hemolytic anemia and idiopathic thrombocytopenic purpura.

Misses Helen Ashenbrucker, Jean Robinson, and Betty Tatting gave technical aid. Dr. M. E. Lahey made serum copper determinations. Miss Mary Lou Helmreich and the staff of the Department of Biochemistry, University of Utah, made determinations of urinary 17-ketosteroids.

41. Thorn and others.[6a] Hench and others.[9b]

42. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K., and Blunt, J. W.: Effect of Cortisone on Production of Granulation Tissue on the Rabbit, Proc. Soc. Exper. Biol. & Med. **72**:718, 1949.

# PRERENAL PROTEINURIA

## I. Particle Size

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WHEN PROTEIN is detected in the urine of patients, we usually find in the urinary sediment direct evidence that a renal lesion exists. During life the development of this lesion can be followed, and after death our inferences concerning the nature of the lesion can be confirmed, corrected and extended by histological examination of the kidney. The disorders of these patients are all instances of what we may call renal proteinuria.

Every now and then, however, we see patients in whose urine protein appears in large amounts, although, at least in the beginning, there is no evidence in the urinary sediment of a renal lesion of any sort. To distinguish these proteinurias from those that develop in association with a renal lesion, we propose to call them "prerenal," this term indicating our supposition that they have their immediate origin in an abnormality, not of the kidney, but of the protein complex of the plasma that perfuses the kidney.

It is to be anticipated that patients with prerenal proteinuria may present themselves with a diversity of clinical features corresponding to the multiplicity of the possible plasma protein abnormalities that might induce proteinuria. Two dissimilar groups are already known. There are the patients with multiple myeloma whose plasma contains a protein that filters readily through the normal glomerular membrane because it has only half the molecular weight of serum albumin. We can confirm Mainzer's observation that in the early stages of this disease there may be no renal lesion.[1] We may thus cite Bence Jones proteinuria as the classic example of a prerenal proteinuria. Recently another instance has appeared. When patients with cirrhosis of the liver are given repeated intravenous injections of large amounts of human serum albumin, a heavy proteinuria appears which cannot be accounted for on the basis of any renal lesion.[2] In this case there is no qualitative defect in the

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This work was made possible by a grant from the United States Public Health Service. Part of it was carried out under a grant from the Committee on Medical Research of the Office of Scientific Research and Development.

1. Mainzer, F.: Klin. Wchnschr. **10**:1906, 1931.

2. Thorn, G. W.; Armstrong, S. H., and Davenport, V. D.: J. Clin. Invest. **24**:304, 1946.

structure of the proteins of the plasma. There is, however, a transient quantitative deformity in the plasma protein structure induced by the injection of albumin. It may be that the proteinuria occurs because the increase in albumin concentration induces such an increase in the usual glomerular filtration of albumin that the tubule cells are no longer able to retrieve all of it, so that some escapes in the urine.

We believe that there are other undiscovered forms of prerenal proteinuria, and that there are clinical reasons, concerned with treatment, which make it important that their mechanisms be comprehended. In the present state of our knowledge, however, all that can be done is to formulate and try to answer certain questions that arise from observation of the course and outcome of prerenal proteinuria in a group of patients who may ultimately be shown to be suffering from widely different forms of plasma protein abnormality. The clinical features they have in common are explicable in terms of certain obvious possibilities. The proteinuria would be accounted for if it were found that the plasma contained small protein molecules that filtered through the glomerular membrane in such number as to exceed the capacity of reabsorption of the tubule cells. Again, even though the plasma proteins were of the usual size and passed in the usual amounts into the glomerular filtrate, the same loss of protein in the urine might occur if these proteins happened to have some structural peculiarity that interfered with their absorption by the proximal tubule cells. Or, still again, both filtration and reabsorption might be intact, but the digestion of the reabsorbed protein might be slowed, either because of some singularity in the pattern of the protein molecule or because of a defect in the intracellular digestive mechanism. This could lead to a glut of undigested protein within the tubule cells that might interfere with further reabsorption and thus give rise to proteinuria.[3] At present these are no more than tenable hypotheses. They are worth mentioning only because it may be possible to affirm or negate each of them by experiments, not on patients, but on animals.

If, for the moment, we disregard the patients with Bence Jones proteinuria and those in whom proteinuria has been induced by the injection of large quantities of serum albumin, there remains a not inconsiderable number in whom from the first the question of the existence of a prerenal form of proteinuria can with reason be raised. A smaller number remains after long observation, and in the end, and after all the evidence is in, there is a still smaller number that, not with certainty, but with a high degree of probability, can be placed in this category. Elsewhere we have summarized the histories of 19 such patients, followed over many years throughout the whole course of their disease.[4] As a rule, they come to us when they are already edematous, and then it may be hard, at least with any assurance, to distinguish them from the larger group with much the same history and presenting not very dissimilar signs who are in the degenerative stage of a glomerular nephritis, the initial stage of which had passed unnoticed. Later on, when we see the proteinuria and edema rather suddenly disappear, usually for no apparent reason, we can be more certain; we can be still more certain if, as sometimes happens, we find

3. Strictly speaking, proteinurias that had their whole origin in a failure of the tubule cells to digest or reabsorb normal proteins that filtered through the glomeruli from a normal plasma would be examples of renal, not of prerenal, proteinuria. For the time being, however, this is purely theoretical. We have no clinical methods that enable us to make this distinction.

4. Addis, T.: Glomerular Nephritis: Diagnosis and Treatment, New York, The Macmillan Company, 1948, p. 142.

a fully recovered patient of this group again displaying proteinuria and edema. As we watch the recurrence it becomes evident that this is an instance of prerenal proteinuria. For then we find that here is a pure proteinuria, without any other manifestation of disease and—the decisive point—with no indication in the urinary sediment of any renal lesion. Not until later, usually after the patient has become edematous, do we find in the sediment the tubule cells, engorged with protein droplets, that are the sign that the capacity of the proximal tubule for the reabsorption or digestion of protein has been overtaxed.

## PRESENT INVESTIGATION

In this paper we report experiments designed to test the possibility that some of the patients in the group whose course we have just outlined may have had protein in their urine because their plasma contained a small, easily filtrable protein component. If that were true, the mechanism of their proteinuria would be like that which characterizes the excretion of Bence Jones protein in patients with multiple myeloma. If we were to succeed in separating the protein from their urine without denaturing it, so that we could dissolve it again and inject it into a rat, we should find that it would filter through normal glomeruli and again would appear in the rat's urine. This is not a new method of investigation. In 1932 Kerridge and Bayliss [5] outlined it as follows:

> We are proceeding to investigate the question as to whether any of the proteins found in human urine in disease are excreted by a normal kidney. Those purified by dialysis from the urine of patients with cardiac lesions and venous congestion do not pass through a normal kidney. Our observations on nephritic urinary proteins are not yet numerous enough to publish.

In theory, at least, this method is capable of extension not only to test the possibility of the existence of small proteins but to validate or disqualify the supposition that some proteinurias may be due to a defect in tubule reabsorption or to a failure to digest the protein the cells have reabsorbed. This is possible because we can contrive experimental conditions under which proteins injected into rats and filtered through the glomeruli can be seen to accumulate within the proximal tubule cells. In a rough way we can measure the quantity reabsorbed by noting the degree of increase in the protein content of the kidney.[6] We should also be able to reveal any gross defect in the process of the digestion of these intracellular masses of reabsorbed protein, because we can watch these masses as they disappear and can even follow the rate of digestion through measurements of the decrease in the weight or, better, in the protein of the kidney.[6] In practice, however, the investigation of these possible reabsorptive or digestive defects in tubule cell function cannot be undertaken with hope of clear results until the technical difficulties in the preparation of considerable quantities of urine proteins have been overcome. This first paper of the series is thus restricted solely to the question whether some instances of prerenal proteinuria arise because plasma proteins smaller than serum albumin are produced. The second and third papers that follow deal with peculiarities in the volume of protein precipitates and in the electrophoretic mobility of proteins obtained from two of the patients in this group.

5. Kerridge, P. M. T., and Bayliss, L. E.: Lancet **2**:785, 1932.

6. Addis, T.: Final Report, Contract 338, Committee on Medical Research, Office of Scientific Research and Development, 1946.

## EXCRETION OF PROTEINS OF KNOWN MOLECULAR WEIGHT THROUGH THE KIDNEYS OF NORMAL RATS

A general conclusion emerges from nearly all the work recently published on the question of excretion of proteins of known molecular weight through the kidneys. It is that proteins with a molecular weight as great as or greater than that of serum albumin (70,000) are not excreted by the kidney, whereas proteins the molecular weight of which is less than 70,000 appear in the urine after they are injected into mammals. Hemoglobin, it is true, occupies an intermediate position, appearing in the urine when its concentration in the plasma exceeds a certain level. However, the fact that the molecular weight of hemoglobin is 68,000 serves to consolidate the view that, as far as the kidney is concerned, there are two distinct classes of proteins, those it does not excrete and those that it does excrete, the reason for the distinction lying in the size of the molecule.

The experimental facts are most simply explained if the glomerular membrane is considered as a filter in which there are holes of a certain size. The holes would be large enough to let a protein of a molecular weight of 34,000 pass, but they would be a rather tight fit for a protein weighing 68,000 and too small to let a protein with a molecular weight of 70,000 through. This hypothesis, because it is the simplest, is very properly the one that has been entertained by some investigators, but further experience has shown us that it will have to be modified. We find, for instance, that we can get the kidneys of a rat to excrete their own weight of serum albumin in 24 hours. They will even excrete a little of a protein as large as gamma globulin. Such observations might be taken as indicating damage to the filter, but that explanation is not satisfactory, for the excretion ceases whenever the injections are stopped and there is no sign of any anaphylactic reaction. Furthermore, a pronounced proteinuria can be induced by simply increasing the concentration of the proteins that already exist in the rat's plasma, as when we inject rat serum. We are, therefore, obliged to believe that all proteins filter through the normal glomerular membrane. The distinction that seems so clear between various proteins in accordance with their size is a true distinction, but it is relative, not absolute. If the particle size of a protein is large, only a little will filter. Then the tubule cells will reabsorb all of it, and none will escape in the urine. If the particle size is small, a large proportion will filter through, and, if the quantity in the filtrate exceeds the reabsorptive capacity of the tubule cells, we shall find it in the urine. But even with a protein of large particle size, we can induce proteinuria if only we inject it repeatedly and in large quantities. The distinction is quantitative, not qualitative.

These general considerations have a direct bearing on the conditions that will have to be observed if we are to succeed in demonstrating the existence of small-sized proteins in the urine of patients. For if the mechanism of proteinuria is as complicated as we suppose—and, of course, in reality it is probably much more complicated than we can now imagine—the mere appearance in the urine of the rat of some protein we have injected cannot be taken as indicating that it has less than the usual molecular weight. An inference concerning molecular size would be justified only if the excretion of the unknown protein was observed in circumstances

that enable us to make this distinction when we inject proteins the molecular weight of which is known and find that under these particular conditions only proteins with a molecular weight appreciably less than 70,000 are excreted.

In order to define the required conditions, we injected varying quantities of proteins of known molecular weight into the peritoneal cavities of groups of six female albino rats whose average body weight was 150 Gm. Each rat was placed in a special cage designed for the collection of urine. After 17 hours the urines of each group were combined for the determination of the rate of protein excretion by a modification of Kingsley's biuret method.[7] During the period of urine collection the animals were given, ad libitum, a 35 per cent solution of dextrose in 0.4 per cent sodium chloride solution, to which was added 1 per cent of a fluid preparation of B complex vitamins. Under these conditions the urine of rats given injections of 10 cc. of 0.85 per cent of sodium chloride solution contains 3.2 mg. of protein per rat per 17 hours. This amount has been subtracted from the rate we report after the injection of 10 cc. of various proteins dissolved in 0.85 per cent sodium chloride solution.

TABLE 1.—*Egg White, Globin and Albumin Excretion by Normal Rats*

| Egg White | | | Globin | | | Albumin | | |
|---|---|---|---|---|---|---|---|---|
| Amount Injected, Mg. | Protein Excreted, Mg./ 17 Hr. | Excretion in Relation to Injection, % | Amount Injected, Mg. | Protein Excreted, Mg./ 17 Hr. | Excretion in Relation to Injection, % | Amount Injected, Mg. | Protein Excreted, Mg./ 17 Hr. | Excretion in Relation to Injection, % |
| 25 | 11 | 44 | 50 | 2 | 4 | 200 | 0 | 0 |
| 50 | 23 | 46 | 100 | 8 | 8 | 800 | 3 | 0 |
| 100 | 48 | 48 | 200 | 16 | 8 | 1,600 | 54 | 3 |

The first proteins we investigated were egg white (molecular weight, 43,000), globin (molecular weight, 65,000) and human serum albumin (molecular weight, 70,000). Egg white is a mixture of proteins of different sizes. In this respect it is like the urine proteins we used. But it does contain egg albumin, the molecular weight of which is usually given as 43,000. Globin was taken as an example of a protein of intermediate size. Pauling,[8] in an investigation into the structure of hemoglobin, has given evidence that there are four heme groups attached to each molecule of globin.[9] If the molecular weight of hemoglobin is 68,000, the globin should weigh 65,000. Substantially the same results were obtained with other albumin preparations he provided that had been modified in various ways.

The results given in Table 1 indicate that under these conditions it should be possible to detect the existence in urine protein of less than 25 mg. of a protein as small as egg albumin, and as little as 100 mg. of a protein-like globin. On the other hand, even 800 mg. of serum albumin produces no significant increase in protein excretion, and, with a single injection, as much as 1,600 mg. is required to produce a substantial proteinuria.

7. Kingsley, G. R.: J. Biol. Chem. **131**:197, 1939.

8. Pauling, L.: Proc. Nat. Acad. Sc. **21**:186, 1935.

9. Dr. M. M. Strumia, Penn Valley, Pa., supplied the globin we used (lot E4944). The "normal" human albumin with which we worked was sent to us by Dr. E. J. Cohn, Cambridge, Mass.

EXCRETION THROUGH THE KIDNEYS OF NORMAL RATS OF PROTEIN FROM THE URINE OF PATIENTS

*Method.*—The urine of patients was collected directly into bottles containing formaldehyde. It was run into seamless cellulose tubing (Visking) which was closed by dental floss ligatures. The tubing with the urine, to which a little toluene had been added, was left overnight in a pail of water that was kept in constant agitation and running over by a stream of water from the cold water tap. Next morning the dialysed urine, now somewhat cloudy because of the precipitation of globulins, was transferred to milk bottles on the sides of which it was frozen by rotation in an alcohol-carbon dioxide mixture. The bottles were then attached to a Flosdorf-Mudd lyophile apparatus, and the vacuum was maintained until the water had been removed. After the rubber connections had been clamped, the bottles were detached and stored at 4 C. until they were needed. Before an injection the dry protein was weighed and dissolved in 0.85 per cent sodium chloride solution, and the protein content was determined. The amount of protein we wanted to inject was then obtained by dilution with salt solution in a volume of 10 ml., which was injected intraperitoneally into each of a group of rats kept under the conditions observed in the experiments on proteins of known molecular weight.

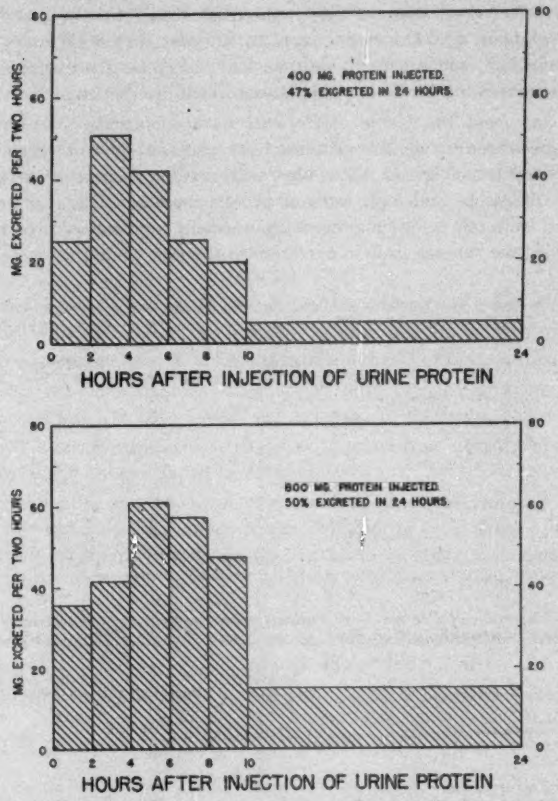
1. *Bence Jones Protein from the Urine of Patients with Myeloma.*—Our first experiment was carried out on protein prepared from the urine of a patient whom, on clinical grounds, we had classified as belonging to the group with prerenal proteinuria. To find whether his urine contained small molecule proteins, we compared the behavior of his protein in the rat with the behavior of protein prepared in the same manner and at the same time from the urine of two patients with multiple myeloma whose 24 hour urine contained 6 to 8 Gm. of Bence Jones protein contaminated with no more than a trace of proteins with other characteristics. Bence Jones protein has a molecular weight of 34,000. It has been observed by Bott and Richards to filter through the glomerular membrane of the frog and to be excreted in the urine of normal dogs.[10] If, therefore, the urine of our patient with prerenal proteinuria contained small protein molecules, we might expect to find an effect on the protein excretion of the rat that was similar to that produced by the injection of the Bence Jones protein.

At that time Dr. Eloise Jameson, Los Angeles, was studying the electrophoretic characteristics of urine proteins. She had precipitated the proteins from these three patients with a potassium citrate-citric acid mixture at pH 6.8. After dialysis, 500 mg. quantities were injected into each of three groups of six female rats maintained on the dextrose solution we have described. At varying intervals after the injection urine was collected, and the protein quantities given in Table 2 were determined.

It is apparent that there is no evidence that the patient with prerenal proteinuria had any appreciable quantity of small molecule protein in his urine. It is clear that the two Bence Jones proteins, though they differed somewhat with respect to the proportions that appeared in the urine, were both rapidly excreted, so that 24 hours after the injection the rate of protein excretion had reverted to about its usual level.

Since the mode of preparation might conceivably alter the particle size by polymerizing or splitting the protein molecules, we later separated Bence Jones protein from the urine of another patient with myeloma, this time following the lyophilization technique that we were then using. The two-hourly rates of excretion from groups of six rats given 400 and 800 mg. of this preparation are shown in the Figure.

10. Bott, P. A., and Richards, A. N.: J. Biol. Chem. **141**:291, 1941.


400 MG. PROTEIN INJECTED.
47% EXCRETED IN 24 HOURS.
MG. EXCRETED PER TWO HOURS.
HOURS AFTER INJECTION OF URINE PROTEIN
800 MG. PROTEIN INJECTED.
50% EXCRETED IN 24 HOURS.
MG. EXCRETED PER TWO HOURS.
HOURS AFTER INJECTION OF URINE PROTEIN

Two-hourly rates of excretion from groups of six rats given 400 and 800 mg. of Bence Jones protein from the urine of a patient with multiple myeloma.

TABLE 2.—*Protein Excreted by Rats During Successive Periods After Injection of 500 Mg. of Urine Proteins from Two Patients with Myeloma and One Patient with Possible Prerenal Proteinuria*

| Period of Collection After Injection, Hr. | Duration of Period of Collection, Hr. | Myeloma (Patient H. G. H.) | | Myeloma (Patient M. E. M.) | | Prerenal Proteinuria (Patient B.) | |
|---|---|---|---|---|---|---|---|
| | | Mg. per Period | Mg. per Hr. | Mg. per Period | Mg. per Hr. | Mg. per Period | Mg. per Hr. |
| 0- 4 | 4 | 53 | 13.3 | 36 | 9.0 | 1 | 0.3 |
| 4- 6 | 2 | 28 | 14.0 | 41 | 20.5 | 1 | 0.5 |
| 6-11 | 5 | 65 | 13.0 | 113 | 22.6 | 2 | 0.4 |
| 11-24 | 13 | 44 | 3.4 | 71 | 5.5 | 6 | 0.5 |
| 24-48 | 24 | 8 | 0.3 | 10 | 0.4 | 5 | 0.2 |
| 48-72 | 24 | 9 | 0.4 | 6 | 0.3 | 6 | 0.3 |
| Total protein excreted in 72 hr., mg. | | 207 | | 277 | | 21 | |
| Protein excreted by controls in 72 hr., mg. | | 13 | | 13 | | 13 | |
| Injected protein excreted, mg. | | 193 | | 264 | | 8 | |
| Proportion of injected protein excreted, % | | 39 | | 53 | | 2 | |

These results reveal that the maximum rate of excretion of a protein with a molecular weight of 34,000 may be expected to occur within 17 hours after intraperitoneal injection, which was the time we had chosen for the examination of proteins prepared from the urines of patients with renal disease.

2. *Proteins from the Urine of Patients with Glomerular Nephritis.*—All 10 patients from whose urines the proteins were prepared were in the degenerative phase of glomerular nephritis. All had low total serum protein concentrations, varying degrees of edema and high rates of protein excretion. Because an occasional animal taken from our colony may have an unusually high rate of protein excretion, we determined the rates of protein excretion in the rats while they were maintained

TABLE 3.—*Excretion of Protein from Patients with Glomerular Nephritis in Normal Rats*

| Patient | Number of Rats | Mg. Injected | Mg. Excreted per 17 Hr. | Excretion in Relation to Injection, % |
|---|---|---|---|---|
| S. | 5 | 200 | 3 | 2 |
| H. | 2 | 200 | 2 | 1 |
| L. | 4 | 400 | 9 | 2 |
| D. | 8 | 400 | 16 | 4 |
| B. | 3 | 400 | 14 | 3 |
| H. | 3 | 400 | 2 | 1 |
| Ho. | 3 | 400 | 7 | 2 |
| Du. | 6 | 400 | 7 | 2 |
| M. | 4 | 800 | 19 | 2 |
| R. | 3 | 800 | 13 | 1 |

TABLE 4.—*Excretion of Protein from Patients with Prerenal Proteinuria in Normal Rats*

| Patient | Number of Rats | Mg. Injected | Mg. Excreted per 17 Hr. | Excretion in Relation to Injection, % |
|---|---|---|---|---|
| Mi. | 4 | 200 | 1 | 1 |
| | 4 | 500 | 1 | 0 |
| L. | 2 | 200 | 4 | 2 |
| | 2 | 400 | 4 | 1 |
| | 2 | 800 | 12 | 2 |
| Bl. | 9 | 400 | 2 | 1 |
| I. | 7 | 400 | 8 | 2 |
| | 7 | 800 | 19 | 2 |
| Be. | 2 | 400 | 3 | 1 |
| P. | 2 | 400 | 1 | 0 |

on dextrose solution a week or so before the protein was injected. We selected for the experiment those rats whose protein excretion was that which is customary. The 17 hour rates given in Table 3 are the actual rates less the 3.2 mg. of protein we found in the urine of the control rats that had been given 10 ml. of salt solution.

If no effect at all had been produced, the average rate of excretion in the group should have approximated 0. The average is, in fact, 9.2 mg. per 17 hours, so that these rats were excreting about four times more than the controls who were given injections of salt solution. However, the proportion of the protein excreted to the protein injected in no instance exceeded 4 per cent. Whatever may be the reason for the slight increase in protein excretion that was produced, we may conclude that in these patients with glomerular nephritis little, if any, of the total proteins of their urine can have been proteins like those that exist in egg white or in the urine of patients with myeloma who have Bence Jones proteinuria.

3. *Proteins from the Urine of Patients with Prerenal Proteinuria.*—Ten experiments were carried out on proteins prepared from the urines of six patients whom we thought belonged to the group with prerenal proteinuria. The clinical features of one of the patients (Bl.) have been described in detail.[11] The results are given in Table 4.

There is nothing to distinguish these patients from those with glomerular nephritis so far as the excretion of their proteins by the rat is concerned. At least in these six patients, then, we can conclude that the proteinuria does not occur because they produce proteins of less than the usual size.

## CONCLUSIONS

By intraperitoneal injection of proteins of known molecular weight it is shown that proteins with the molecular weight of serum albumin, as well as proteins of lesser molecular weight, may be excreted by the kidneys of normal rats.

Conditions are defined under which only those injected proteins that have a molecular weight of less than 70,000 are excreted in the urine of the normal rat.

A method is described for the separation of protein from urine in an undenatured form suitable for parenteral injection into animals.

Proteins prepared from the urine of patients classified as belonging to the group in which proteinuria occurs because of abnormalities in their plasma protein structure (prerenal proteinurias) were shown to contain no appreciable amount of small protein molecules unless the urines from which they were derived contained Bence Jones protein.

Proteins prepared from the urine of patients with glomerular nephritis contained no appreciable amount of small protein molecules.

Dr. Jessie Marmorston assisted in the preparation of these papers for publication.

11. Addis, T.: Stanford M. Bull. 3:67, 1945.

# PRERENAL PROTEINURIA

## II. Observations on the Urinary Protein

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ADDIS has described [1] the clinical course of a supposed disorder of plasma protein metabolism, frequently cyclic, which is characterized initially by abnormal formation of plasma protein and proteinuria, following which a degenerative type of renal lesion develops, the plasma protein concentration falls and edema appears. When the patient he first described [1a] was well, the plasma protein electrophoretic pattern was not unusual. The protein from both blood and urine produced an abnormal pattern, however, when the above noted findings were present. At pH 7.7 the fraction which had the mobility of serum albumin was split in two.[2] The hypothesis was advanced that the primary defect in this syndrome is both quantitative and qualitative, with a reduction in the rate of plasma protein formation and a production of abnormal protein molecules. It is supposed that the proteinuria occurs because of a structural defect in the plasma protein molecules, and that the renal lesion develops as a consequence of the proteinuria. In the first paper of this series, Addis and his associates [3] presented information on the size of the urinary protein molecules of the patient studied in these experiments, and in the third paper Jameson and Addis[2] give electrophoretic data on this patient. The experiments reported here represent an attempt to determine by another technique whether or not the urinary protein from patients with prerenal proteinuria differs from that found in the urine of patients with glomerular nephritis.

The biuret reaction for protein depends on the presence of polypeptide linkages and, thus, presumably is not influenced by molecule size or shape. According to Kingsley,[4] equal quantities of albumin and globulin yield violet colors of the same intensity. On the other hand, I shall show that the volume of precipitated protein obtained under standard conditions is a unique characteristic of each prepared

These experiments were aided by a grant from the Columbia Foundation.

From the Department of Medicine, Stanford University School of Medicine.

1. (*a*) Addis, T.: Renal Degeneration Due to Protein Reabsorption by the Kidney, Stanford M. Bull. **3**:67, 1945; (*b*) Glomerular Nephritis, New York, The Macmillan Company, 1948.

2. Jameson, E., and Addis, T.: Prerenal Proteinuria: III. Electrophoretic Studies, this issue, p. 350.

3. Addis, T.; Barrett, E.; Poo, L. J., and Ureen, H.: Prerenal Proteinuria: I. Particle Size, this issue, p. 337.

4. Kingsley, G. R.: The Determination of Serum Total Protein, Albumin and Globulin by the Biuret Reaction, J. Biol. Chem. **131**:197, 1939.

protein investigated. The observations on eight known prepared proteins indicate that solutions of equal concentrations of these proteins produce different volumes of precipitate, and that the volume is not dependent on molecule size. It was thought, therefore, that a comparison of values derived from protein determinations on the same urine specimens by methods involving both the biuret reaction and the volume of precipitated protein might give some evidence for the existence of proteins in the urine of patients with prerenal proteinuria which are not present in the urine of patients with glomerular nephritis.

## METHODS

In the precipitate method used here, protein is precipitated and centrifuged under conditions in which the time and rate of speed of centrifugation are controlled. The volume of packed protein precipitate is corrected for temperature and is converted into grams of protein by reference to a table. The table used for the studies on the patient's urine was prepared by relating volume of precipitated protein and protein content as determined by the biuret method. The biuret colors had been standardized by comparing them with results obtained by weighing thoroughly washed and dried urine protein from patients with glomerular nephritis. A similar table, based on rat urine, which contains small amounts of protein, was employed for the experiments on the prepared proteins. The rat urine table was used for these studies because at the time experiments involving the rates of excretion of the same prepared proteins from rats were being undertaken. Separate tables for human and rat urine were necessary, as dissimilar proteins, which produce different volumes of precipitate, are found in these urines. These methods of precipitation were originated for the purpose of obtaining a method for the rapid determination of the rate of protein excretion in patients.[1b] The biuret method used was a modification of that described by Kingsley.[4] The protein content of each specimen was determined by both methods. All tests were made in duplicate.

Eight prepared proteins were investigated. These were human albumin, egg white, bovine albumin, bovine fractions I (mainly fibrinogen), II (mainly gamma globulin) and III (mainly beta globulin), human gamma globulin and Bence Jones protein.

During these experiments, the patient reported on by Addis [1a] was excreting large quantities of protein, and his condition conformed to the syndrome previously noted. Fifty-six separately voided urine specimens from this patient were tested. Similar examinations were made of 79 separately voided urine specimens from four patients in the degenerative stage of glomerular nephritis.

Values for the protein content of these specimens, as determined by the biuret method and the precipitate method, were compared graphically (Figs. 1 and 2). The data from the patients were submitted to statistical analysis; regression lines were drawn, and the significance of the difference between these lines was determined.

## RESULTS AND COMMENT

*Prepared Proteins.*—The volumes of protein precipitate produced from the prepared protein solutions of equal concentrations differed greatly (Fig. 1). No direct relationship appears to exist between molecular weight and the volume of protein precipitate. Preparations containing proteins of similar molecular weight, such as egg white and Bence Jones protein, may yield markedly different volumes of precipitated protein. Proteins of higher molecular weight (i. e., human gamma globulin with a molecular weight of 150,000) may produce smaller volumes of precipitate than proteins the molecular weight of which is less (i. e., bovine albumin with a molecular weight of 70,000).

*Patient Proteins.*—The relationship between the results obtained by the precipitate method and by the biuret method for the urinary protein from the patient with prerenal proteinuria differs from that noted for the urinary protein from patients

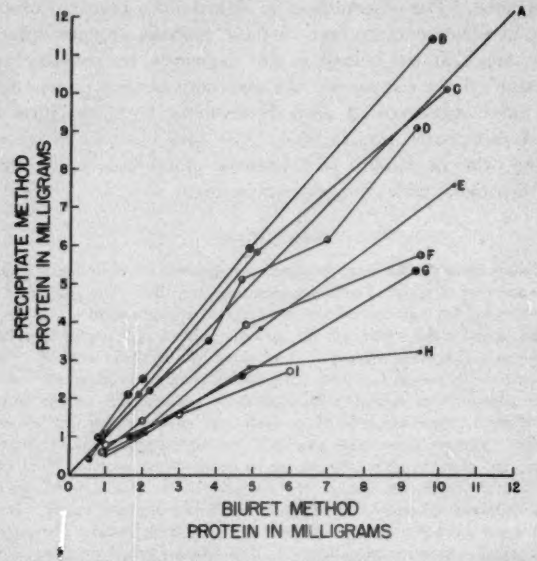


Fig. 1.—Lines of method relationship. *A*, rat urinary protein. *B*, bovine albumin (molecular weight, 70,000). *C*, human albumin (molecular weight, 70,000). *D*, egg white (molecular weight, 34,000). *E*, bovine fraction II (molecular weight, 176,000). *H*, Bence Jones protein (molecular weight, 34,000). *I*, bovine fraction I (molecular weight, 500,000).

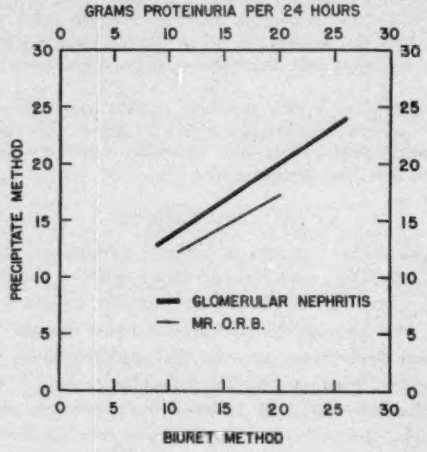


Fig. 2.—Method relationship for urinary protein from patients with glomerular nephritis and for urinary protein from a patient with prerenal proteinuria (Mr. O. R. B.).

with glomerular nephritis. When this difference is compared statistically, the visual impression is confirmed, for the difference is significant.[5]

Neither the magnitude of this dissimilarity nor the relative positions of the regression lines are direct reflections of molecule size, if there is no direct relationship between molecule size and the volume of precipitated protein. Addis and his co-workers,[3] furthermore, have demonstrated that the molecules of urinary protein from the patient studied here were not small in size. These data indicate that the urinary protein from the patient with prerenal proteinuria differed from the urinary protein of the patients with glomerular nephritis. What this difference consists of is not clear, but it is reasonable to suppose that differences in hydration of the protein molecules are involved.

## SUMMARY

The protein content of eight known protein preparations was determined by the precipitate method and by the biuret method. No direct relationship between the volume of packed precipitated protein and molecular weight was noted. Each prepared protein produced a specific and different volume of protein precipitate under standard conditions.

The urinary proteins from a patient with prerenal proteinuria and from four patients in the degenerative stage of glomerular nephritis were studied similarily. The urinary protein from the patient with prerenal proteinuria differed from that produced by the patients with renal proteinuria, forming a smaller volume of packed protein precipitate under standard conditions.

Mrs. Evalyn Barrett and Miss H. J. Ureen assisted in this study.

5. The equation for the values from the patients with glomerular nephritis was $Y = 7.10 - 0.654X$, and that for the patient with prerenal proteinuria was $Y = 6.48 - 0.625X$, where $X$ equals grams of protein in the urine per 24 hours by the biuret method and $Y$ equals grams of protein in the urine per 24 hours by the precipitate method. Comparison of these showed that the slopes are not statistically different. The levels, however, are markedly different, since the difference between the intercepts (mean $Y$) divided by the standard error of the difference was 12, corresponding to the small probability of 0.001, i. e., 1 chance in 1,000 that such a difference might arise fortuitously.

Dr. Horace Gray performed the statistical analysis of these data.

# PRERENAL PROTEINURIA

## III. Electrophoretic Studies

ELOISE JAMESON, Ph.D.
AND
T. ADDIS, M.D.†
SAN FRANCISCO

ELECTROPHORETIC studies were carried out on the proteins from serum and urine of patients who suffered from what has been described previously as prerenal proteinuria.[1] In each case the rate of protein excretion was very large. Two patients were followed for six months or more during both exacerbation and remissions of their disease. During episodes of proteinuria, two albumins, split globulins and a low gamma globulin were found in either the serum or the urine, and sometimes in both. The reappearance of the albumin as a single fraction was the first sign of recovery from the proteinuria, the split globulin persisting until the edema was gone. Abnormalities of the electrophoretic pattern of the serum proteins of the one patient studied in this respect were lessened by the addition of human gamma globulin to the serum.

### EXPERIMENTAL METHODS AND RESULTS

*Method.*—The serum to be studied was diluted to about 1.25 per cent protein in the usual manner with Cohn's phosphate buffer, pH 7.7, or Ringer's solution, pH 7.4. This pH was used so that physiological conditions could be maintained as well as possible. Longsworth's modification of the Tiselius method was used.

*Results.*—The serum and urine from the first patient with prerenal proteinuria (Mrs. M.) were observed both during an attack of unexplained proteinuria and during recovery (fig. 1 and table 1). The proteinuria was accompanied with changes in the serum, chief of which was the presence of a low gamma globulin and a split albumin. Split beta and gamma globulins persisted during recovery long after the proteinuria had ceased and until the edema disappeared. The constituents of urinary protein prepared dry through lyophilization are given in table 2 and figure 2. The preponderance of this urinary protein was found to be homogeneous albumin.

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Dr. Jameson is now at the University of Southern California, Los Angeles.

†Dr. Addis' death occurred on June 4, 1949, before this paper was completed.

From the Department of Medicine, Stanford University, San Francisco, and Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles.

1. Addis, T.: Stanford M. Bull. **3**:67, 1945; Glomerular Nephritis, New York, The Macmillan Company, 1948.

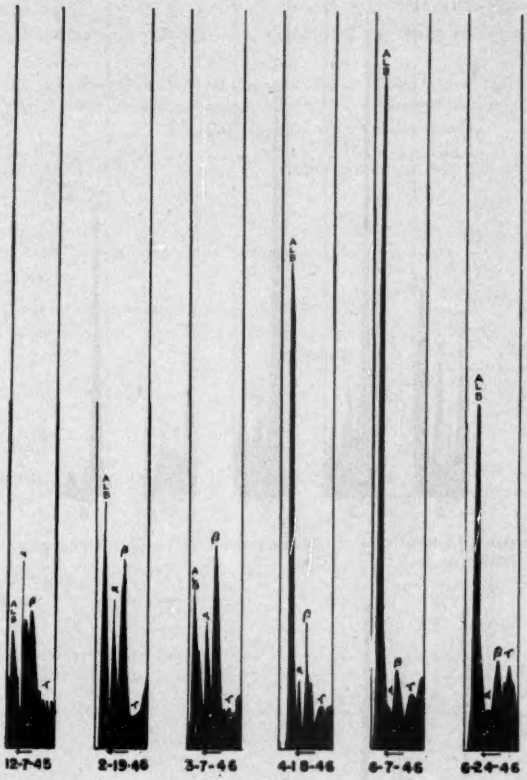


Fig. 1.—Electrophoretic patterns of serum proteins during an attack of prerenal proteinuria and recovery.

TABLE 1.—*Electrophoretic Analyses of Proteins in Serum (Mrs. M.)*

| Date | Buffer Solution pH | Per Cent of Albumin | Per Cent of Globulins | | |
|---|---|---|---|---|---|
| | | | Alpha | Beta | Gamma |
| 12/ 7/45 * | Ringer's 7.47 | 28.1 † | 38.0 | 27.4 | 6.5 † |
| 12/10/45 * | Cohn's 7.7 | 23.6 † | 34.5 | 29.9 | 11.8 |
| 3/ 7/46 * | Cohn's 7.7 | 42.5 † | 18.7 | 33.7 † | 5.1 † |
| 4/18/46 | Ringer's 7.4 | 64.1 | 11.0 | 12.9 | 12.0 |
| 4/18/46 | Cohn's 7.7 | 58.2 | 8.9 | 19.0 † | 13.9 |
| 6/ 7/46 | Ringer's 7.4 | 67.3 | 5.6 | 14.5 † | 12.7 |
| 6/21/46 | Ringer's 7.4 | 62.3 | 3.8 | 16.1 † | 17.7 |
| 6/21/46 | Ringer's 7.4 | 61.2 | 4.9 | 17.7 | 16.2 † |

* Proteinuria and edema are present. On March 7, 1946 the 24 hour excretion of protein was 10.1 Gm. After March 7, 1946 the proteinuria had ceased and the edema decreased, until on June 21, 1946 it was practically absent.

† Split protein peaks. Averages of ascending and descending sides. See figure 1 for electrophoretic patterns.

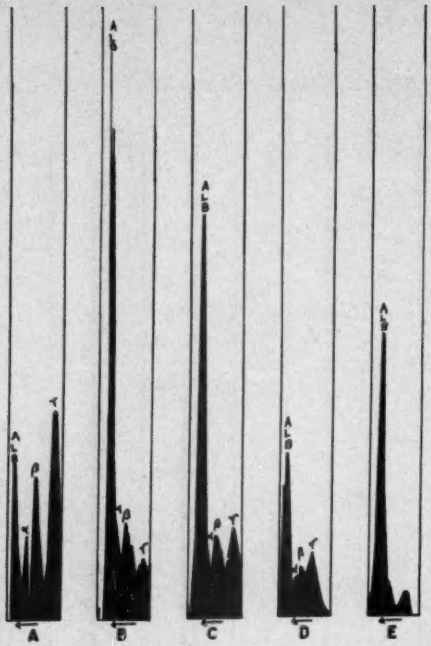


Fig. 2.—Electrophoretic patterns of urinary protein (Mrs. M.) during an attack of prerenal proteinuria and recovery.

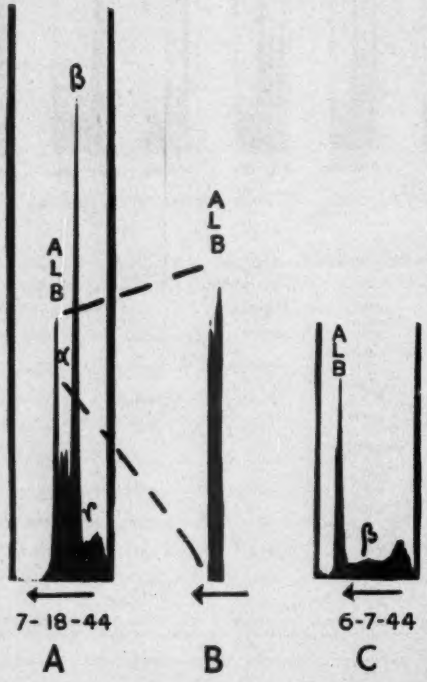


Fig. 3.—Electrophoretic patterns of serum and urinary proteins during an attack of prerenal proteinuria. *A*, serum pattern, ascending side. *B*, enlarged tip of albumin peak in *A*. *C*, urinary proteins, ascending side.

Table 3 and figure 3 show the split fractions which were found in another patient (Mr. B.) with prerenal proteinuria. As long as the split fractions remained in

TABLE 2.—*Electrophoretic Analyses on Human Dried Urine (Mrs. M.)*

| Buffer Solution, pH | Averages of Ascending and Descending Sides, Per Cent of | | |
|---|---|---|---|
| | Albumin | Alpha Globulin | Beta Globulin |
| Cohn's phosphate 7.7 | 81.3 | 10.9 | 7.9 |

The albumin is homogeneous. There is no visible gamma globulin.

TABLE 3.—*Electrophoretic Analyses of Human Serums (Mr. B.)*

| Date | Per Cent of | | | |
|---|---|---|---|---|
| | Albumin | Alpha Globulin | Beta Globulin | Gamma Globulin |
| 10/19/43 | 35.8 * | 15.5 | 40.4 | 8.4 |
| 3/ 7/44 | 54.0 | 4.9 | 31.3 | 9.9 |
| 6/12/44 | 46.1 * | 10.4 | 8.2 * 23.1 | 12.2 |
| 6/14/44 | 48.1 | 13.5 * | 25.0 * | 13.4 |
| 7/18/44 | 32.4 * | 10.2 | 48.8 | 8.5 |
| 7/21/44 | 28.6 | 12.1 | 54.8 * | 4.5 |
| 9/16/44 | 41.6 * | 10.9 * 15.6 | 15.4 | 7.5 |

* Split peaks of proteins.
Cohn's phosphate buffer, pH 7.7, was used.

TABLE 4.—*Electrophoretic Analyses of Human Urine (Mr. B.)*

| Date | Per Cent of | | | |
|---|---|---|---|---|
| | Albumin | Alpha Globulin | Beta Globulin | Gamma Globulin |
| 10/19/43 | 84.5 * | .... | 10.1 | 5.4 |
| 6/ 7/44 | 75.2 * | 14.2 | 10.6 | .... |
| 6/21/44 | 74.8 | 11.2 | 11.0 | 3.1 |
| 7/15/44 | 85.9 | 8.1 | 6.1 | .... |
| 7/18/44 | 89.3 † | 1.3 | 5.7 | 3.8 |

* Split peaks.
† Negative side only.
Cohn's phosphate buffer, pH 7.7, was used.

TABLE 5.—*Electrophoretic Analyses of Serum Proteins in Other Prerenal Proteinurias*

| Patient | Per Cent of | | | |
|---|---|---|---|---|
| | Albumin | Alpha Globulin | Beta Globulin | Gamma Globulin |
| J. | 20.3 * | 42.4 | 37.3 | .... |
| R. | 20.0 * | 13.0 | 60.0 | 7.0 |
| M. | 38.9 | 17.9 | 29.6 | 13.6 |
| N. | 39.5 | 19.0 | 19.7 | 21.8 |

* Split peaks of proteins.

either serum or urine, the edema persisted. Table 4, which gives analyses of this patient's urinary protein, also indicates essentially similar findings.

Tables 5 and 6 give the electrophoretic analyses of protein from the serums and urines of other patients whose condition was classified as prerenal proteinuria by Addis. Split albumins appeared either in the serum or in the urine, or in both, of each patient.

*Addition of Human Gamma Globulin to Mrs. M.'s Serum.*—Alcohol-precipitated human gamma globulin (Cohn's fraction II[2]) was added to four different samples of the patient's serum. The results are shown in table 7 and figure 2. The addition

TABLE 6.—*Proportions of Urinary Proteins in Other Prerenal Proteinurias*

| Patient | Per Cent of | | | |
|---|---|---|---|---|
| | Albumin | Alpha Globulin | Beta Globulin | Gamma Globulin |
| J. | 83.5 * | 11.1 | 4.3 | 2.1 |
| R. | 86.0 * | .... | 7.2 | 6.8 |
| M. | 92.3 * | 2.5 * | 5.2 | .... |
| N. | 82.7 * | .... | 7.5 | 9.8 |

* Split peaks of protein.

TABLE 7.—*Electrophoretic Analyses: Changes in Proportions of Other Fractions After the Addition of Human Serum Gamma Globulin**

| Date | Buffer Solution pH | Per Cent of | | | | | |
|---|---|---|---|---|---|---|---|
| | | Albumin | | Alpha Globulin | | Beta Globulin | |
| | | Before Addition of Gamma Globulin | After Addition of Gamma Globulin | Before Addition of Gamma Globulin | After Addition of Gamma Globulin | Before Addition of Gamma Globulin | After Addition of Gamma Globulin |
| 2/14/46 | Cohn's phosphate 7.7 | 39.5 | 38.1 | 23.3 | 24.0 | 37.3 | 37.9 |
| 3/ 7/46 | Cohn's phosphate 7.7 | 44.8 | 40.0 | 18.8 | 21.1 | 35.5 | 39.0 |
| 6/ 7/46 | Ringer's 7.4 | 76.7 | 71.2 | 7.4 | 10.1 | 15.8 | 18.7 |
| 6/25/46 | Ringer's 7.4 | 73.0 | 67.6 | 5.7 | 11.1 | 21.2 | 21.2 |

* Figures exclude gamma globulin.

of gamma globulin to serum containing split albumin produced a homogeneous albumin. The split beta globulins in experiments on March 7 and June 7, 1946 (see fig. 1) became single fractions after addition of the gamma globulin. The proportions of the proteins (which exclude gamma globulin), as presented in table 7, showed a decrease in albumin, suggesting that the abnormal part of the albumin had become attached to the added gamma globulin, and an increase of the alpha and beta globulins. The appearance of the albumin and alpha and beta globulins approached that in normal serum, although the albumin was low on Feb. 14 and March 7, 1946. In the case on Feb. 14, 1946, the visible edge of the lipids had been changed from the peak of the alpha globulin to the beta globulin by the addition of gamma globulin.

2. Provided by Dr. E. J. Cohn, Cambridge, Mass.

*Effect of the Injection of the Patient's Serum (With and Without Added Gamma Globulin) on the Rat Serum Pattern.*—Mrs. M.'s serum was collected during a period of proteinuria. An electrophoretic pattern was not made because of the small amount of material. Rats were given intraperitoneal injections of 4.1 ml. The rat serum subsequently showed split beta and gamma globulins (fig. 2).[3]

TABLE 8.—*Electrophoretic Analyses of Pleural Fluid of Rats*

| Material Injected | Solution pH | Per Cent * of Albumin | Alpha Globulin | Beta Globulin | Gamma Globulin |
|---|---|---|---|---|---|
| Patient's serum ..... | Ringer's 7.4 | 17.6 † 37.2 | 17.4 | 25.6 | 2.4 |
| Patient's urine ..... | Ringer's 7.4 | 61.8 19.4 | ..... | 7.9 | 11.0 |

* Averages of ascending and descending sides.
† Proportions of the albumins are reversed in the two experiments.

## COMMENT

The importance of gamma globulin and its probable relationship to the other serum proteins were discussed in a previous communication.[4] The electrophoretic patterns of the serum and urinary proteins from several patients whose disorder was classified as prerenal proteinuria (table 5) showed split albumins, as well as split peaks of alpha, beta and gamma globulins. The addition of gamma globulin to the serum from one of these patients resulted in the protein becoming homogeneous, with disappearance of the split peak. The lipids appeared to be attached in a more normal way to the beta globulin after gamma globulin had been added to the serum. The physiological effects caused by the injection of the abnormal serums into rats was prevented by the addition of gamma globulin before injection. In view of these facts, it seems likely that some cementing or solubilizing substance present in normal serum might be lacking in the serum of patients with prerenal proteinuria. It is suggested that it may be present in the gamma globulin normally. The experiments reported here give support to this hypothesis.

## CONCLUSION

Protein derived from serum and urine of patients with prerenal proteinuria differs from that found in normal persons. Studied electrophoretically, the protein from both urine and serum was found to contain split albumin fractions, split globulins and low gamma globulin concentration. It is suggested that these changes may be due to the lack of some solubilizing or cementing substance present normally in the serum.

3. The rat's pleural cavity contained fluid after the patient's serum had been injected. The addition of the gamma globulin to the serum before injection prevented the formation of the pleural fluid. Analyses of the pleural fluid obtained from rats after injection of Mrs. M.'s serum, and of the pleural fluid after injection of her dried urinary protein, are given in table 8. Both have two albumins. See figure 2.

4. Jameson, E.: Arch. Biochem. **15**:389, 1947.

# USE OF MIXTURES OF NPH AND UNMODIFIED INSULINS

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WITH THE release of NPH insulin for sale on the open market, details concerning its action become of importance to physicians and patients. This new modified protamine insulin is an "intermediate insulin," with a duration of action longer than that of unmodified (crystalline or regular) but shorter than that of protamine zinc insulin.[1] NPH insulin, however, resembles protamine zinc insulin in its action much more closely than it does unmodified insulin.

The effect of NPH insulin lasts 26 to 30 hr. in the average nonfasting diabetic patient, and the intensity of action is greater than that of protamine zinc insulin at any given time. Thus, patients who previously required an injection of crystalline [2] insulin along with that of protamine zinc insulin may now, in certain instances, achieve comparable control of diabetes with a single injection of the NPH variety. In certain less easily controlled patients taking NPH insulin, a period of hyperglycemia may occur in the forenoon in spite of a normal fasting blood-sugar level. Obviously, for patients showing this pattern, a sharper effect than can be obtained by NPH insulin alone is desired. In these patients a dose of crystalline insulin should be given along with that of the NPH insulin. When this is done, however, the patient is deprived of one of the advantages of NPH insulin, namely, a reduction in the number of daily injections. It was decided, therefore, to compare the effect of NPH and crystalline insulins when mixed in a syringe with that of comparable doses given by separate injections. The studies to be reported were carried out at a summer camp for diabetic boys.

## CLINICAL MATERIAL AND METHODS

The manner in which the Elliott P. Joslin Camp for diabetic boys is operated has been described elsewhere.[3] During the summer of 1950, 132 boys were cared for. After each boy

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From the George F. Baker Clinic, Elliott P. Joslin, M.D., Medical Director, New England Deaconess Hospital.

The expenses of this study were borne in part by The Diabetic Fund. NPH insulin was furnished by Eli Lilly & Company, Indianapolis 6.

1. Peck, F. B.: Insulin Mixtures and Modifications, Proc. Am. Diabetes A. (1946) **6**:273-300, 1947.

2. Crystalline insulin was used in this study as the rapidly acting, unmodified variety. For all practical purposes, "regular" insulin may be used in place of crystalline insulin.

3. Gabriele, A. J., and Marble, A.: Clinical Experience with a New Modified Protamine-Insulin (NPH-50), Am. J. Digest. Dis. **16**:197-206 (June) 1949; Experiences with 116 Juvenile Campers in a New Summer Camp for Diabetic Boys, Am. J. M. Sc. **218**:161-171 (Aug.) 1949.

was admitted to camp, he received a physical examination, was given an appropriate diet, and was continued on the insulin regimen he had used at home. Our first endeavor was to bring each camper's diabetes under control as quickly as practicable. Control was usually achieved readily, and marked reduction of the daily insulin dosage was frequently necessary. This required waiting at least a week before choosing subjects for the study. During this preliminary period, it was found advisable in certain instances to shift the camper's insulin from one type to another or to a combination of insulins. When our objective was finally achieved, we chose certain cooperative subjects from a group of volunteers whose diabetes appeared to be controlled by the combined use of crystalline and NPH insulins. These boys were 8.8 to 16.7 yr. in age and had had diabetes 0.6 to 12.8 yr. In most, the diabetes was of severe grade. The amount of insulin required in this group ranged from 4 units of crystalline insulin plus 10 units of NPH insulin to 18 units of crystalline insulin plus 48 units of NPH insulin. The period in camp for each subject varied from two to nine weeks.

The entire 24-hr. output of urine was collected daily and tested for the presence of acetone and sugar. The percentage of sugar in the urine was determined quantitatively by the Exton modification[4] of the Sumner method,[5] adapted for use with a Leitz photoelectric colorimeter. Single specimens of urine were collected daily at 7:15 and 11:15 a. m. and at 4:45 and 7:45 p. m. and tested qualitatively for sugar with Benedict's solution. A few minutes before passing each of these specimens, the camper voided in order to empty his bladder and to eliminate error due to postprandial glycosuria. Capillary blood-sugar studies were done twice weekly. The blood was drawn at 7 a. m. and every two hours thereafter, to and including 9 p. m., and again the following morning at 7 a. m. The blood sugar was determined by Folin's method,[6] adapted for use with a Leitz photoelectric colorimeter.

Insulin was administered 30 to 45 min. before breakfast each day. A midafternoon feeding of approximately 20 gm. of carbohydrate (eight "saltines") was given to each camper. The only variable in the total diet for the day was in the amount of carbohydrate given as a bedtime feeding. This feeding was based on the result of the 7:45 p. m. urine test and consisted of 10 to 14 gm. of carbohydrate, 8 gm. of protein, and 14 gm. of fat, in the form of crackers and peanut butter.

## PLAN OF STUDY

All together, 23 boys were studied, though there were never more than 10 boys at any given time. During the study one-half of the group received separate injections of crystalline and NPH insulins, while the other half received the required amounts of the two insulins mixed in a syringe and given as a single injection. Every four to six days the groups were reversed so that those previously receiving separate injections were given daily a single injection of the two insulins mixed in the syringe, and the group which had been having a single injection of the combined insulins now received two injections a day. This procedure was continued throughout the period of study. The day before the groups were reversed, capillary blood-sugar determinations were made every two hours as described earlier. This pattern was adhered to until the last three weeks of camp. During the latter period the capillary blood-sugar tests were made on two successive days each week. The groups were reversed weekly on the morning of the second day that the blood-sugar studies were carried out. With the use of the latter method, it was possible for each boy to receive the same insulin dose on each of the two successive days. More frequently, too, the fasting blood-sugar levels were closely approximated, making comparison of the two regimens more favorable.

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4. Exton, W. G.: Diabetes and Bright's Disease as Selection Problems, read before the Medical Section at the 28th Annual Meeting of the American Life Convention, June, 1938.

5. Sumner, J. B., and Graham, V. A.: Dinitrosalicylic Acid: A Reagent for the Estimation of Sugar in Normal and Diabetic Urine, J. Biol. Chem. **47**:5-9 (June) 1921. Summer, J. B.: A More Specific Reagent for the Determination of Sugar in Urine, ibid. **65**:393-395 (Sept.) 1925.

6. Folin, O., and Malmros, H.: An Improved Form of Folin's Micro Method for Blood Sugar Determinations, J. Biol. Chem. **83**:115-120 (July) 1929.

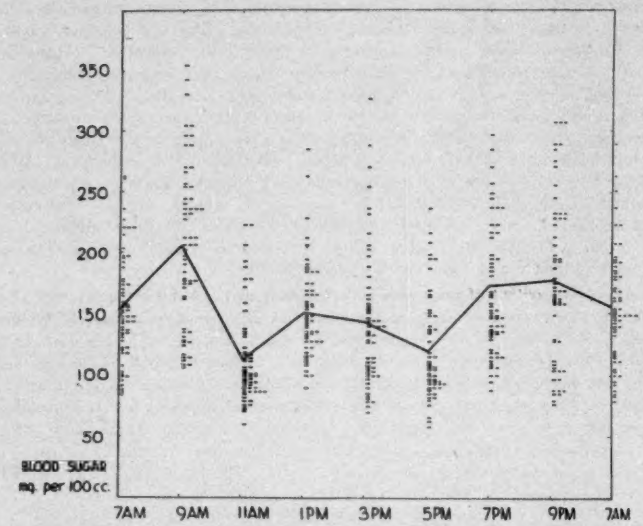


Fig. 1.—Scattergram of capillary blood-sugar values obtained after separate injections of NPH and crystalline insulins.

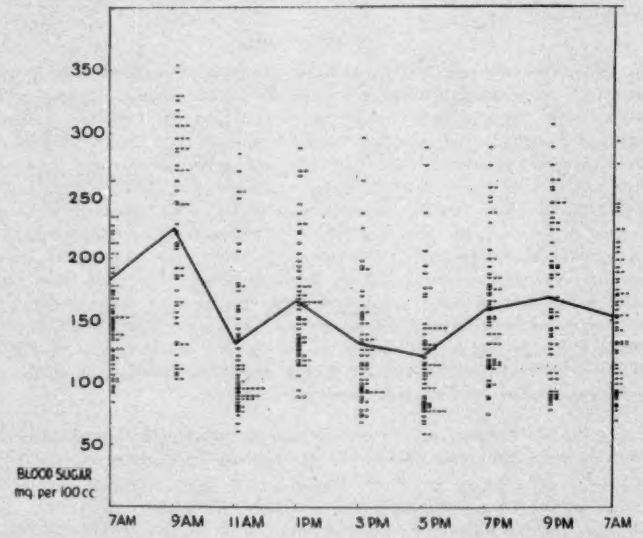


Fig. 2.—Scattergram of capillary blood-sugar values obtained after injections of mixtures of NPH and crystalline insulins in a syringe.

*Summary of Data on Eleven Boys with Diabetes, Showing Response of Capillary Blood Sugar to Mixtures and to Separate Injections of Comparable Doses of Crystalline and NPH Insulin*

| Case No. | Age, Yr. | Duration of Diabetes Mellitus, Yr. | Height, In. | Weight, Lb. | Diet: Carbohydrate, Gm. | Diet: Protein, Gm. | Diet: Fat, Gm. | Diet: Calories | Insulin: Crystalline | Insulin: NPH | Blood Sugar, Mg. per 100 Cc.: 7 A.M. | 9 A.M. | 11 A.M. | 1 P.M. | 3 P.M. | 5 P.M. | 7 P.M. | 9 P.M. | 7 A.M. | Date |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 13.3 | 6.1 | 63.1 | 112 | 216 | 128 | 127 | 2,519 | **10*** | **46*** | 157 | 157 | 111 | 165 | 85 | 81 | 116 | 82 | 103 | July 31 |
| | | | | | | | | | 10 | 46 | 122 | 197 | 131 | 144 | 101 | 81 | 165 | 87 | 148 | Aug. 4 |
| 45 | 12.1 | 1.3 | 58.0 | 87 | 226 | 108 | 109 | 2,317 | 4 | 10 | 148 | 174 | 122 | 131 | 146 | 165 | 194 | 171 | 160 | July 24 |
| | | | | | | | | | **4** | **10** | 127 | 100 | 106 | 122 | 97 | 99 | 131 | 192 | 90 | July 31 |
| | | | | | | | | | 4 | 10 | 133 | 106 | 92 | 127 | 99 | 99 | 164 | 96 | 144 | Aug. 4 |
| | | | | | | | | | **4** | **10** | 145 | 114 | 104 | 128 | 127 | 144 | 236 | 228 | 124 | Aug. 9 |
| | | | | | | | | | **6** | **40** | 97 | 165 | 87 | 89 | 112 | 78 | 87 | 87 | 131 | Aug. 22 |
| 129 | 14.7 | 9.1 | 59.2 | 96 | 216 | 126 | 128 | 2,520 | 6 | 40 | 131 | 131 | 72 | 165 | 144 | 84 | 99 | 111 | 78 | Aug. 23 |
| 96 | 16.3 | 4.1 | 66.2 | 140.5 | 228 | 129 | 127 | 2,571 | 4 | **48** | 127 | 254 | 139 | 177 | 139 | 96 | 165 | 194 | 111 | Aug. 4 |
| | | | | | | | | | 4 | 48 | 148 | 177 | 90 | 133 | 127 | 92 | 114 | 87 | 137 | Aug. 9 |
| | | | | | | | | | **4** | **48** | 126 | 192 | 114 | 137 | 68 | 59 | 114 | 105 | 158 | Aug. 22 |
| | | | | | | | | | 4 | 48 | 158 | 212 | 137 | 206 | 134 | 186 | 256 | 274 | 184 | Aug. 23 |
| | | | | | | | | | 4 | 46 | 129 | 212 | 106 | 156 | 127 | 101 | 150 | 174 | 153 | Aug. 29 |
| | | | | | | | | | **4** | **46** | 153 | 152 | 61 | 183 | 112 | 131 | 184 | 168 | 171 | Aug. 30 |
| 108 | 11.9 | 1.8 | 64.2 | 97 | 226 | 108 | 109 | 2,317 | 4 | 20 | 118 | 212 | 94 | 116 | 133 | 92 | 194 | 206 | 153 | Aug. 22 |
| | | | | | | | | | **4** | **20** | 152 | 170 | 97 | 144 | 95 | 88 | 190 | 220 | 197 | Aug. 23 |
| 123 | 13.3 | 6.8 | 65.0 | 103 | 226 | 127 | 127 | 2,555 | **6** | **34** | 114 | 244 | 158 | 212 | 73 | 67 | 148 | 131 | 153 | Aug. 22 |
| | | | | | | | | | 6 | 34 | 153 | 270 | 152 | 89 | 114 | 127 | 184 | 157 | 162 | Aug. 23 |
| 97 | 14.1 | 2.8 | 64.5 | 115 | 226 | 108 | 109 | 2,317 | 6 | 20 | 118 | 116 | 87 | 116 | 78 | 58 | 135 | 75 | 168 | Aug. 23 |
| | | | | | | | | | **6** | **20** | 168 | 208 | 74 | 148 | 75 | 70 | 74 | 184 | 171 | Aug. 22 |
| | | | | | | | | | **6** | **20** | 178 | 184 | 81 | 127 | 96 | 82 | 131 | 93 | 153 | Aug. 29 |
| | | | | | | | | | 6 | 20 | 153 | 232 | 70 | 127 | 82 | 75 | 148 | 148 | 170 | Aug. 30 |
| 102 | 16.7 | 11.8 | 68.5 | 126 | 230 | 136 | 133 | 2,651 | 4 | 44 | 84 | 184 | 81 | 127 | 152 | 81 | 118 | 103 | 103 | Aug. 15 |
| | | | | | | | | | **4** | **44** | 103 | 174 | 87 | 135 | 99 | 73 | 101 | 122 | 108 | Aug. 16 |
| | | | | | | | | | **4** | **44** | 118 | 186 | 96 | 152 | 89 | 144 | 186 | 228 | 170 | Aug. 23 |
| | | | | | | | | | 4 | 44 | 139 | 128 | 87 | 184 | 114 | 93 | 114 | 168 | 212 | Aug. 29 |
| 101 | 14.8 | 3.1 | 66.7 | 129 | 230 | 138 | 132 | 2,660 | **12** | **40** | 188 | 288 | 144 | 152 | 103 | 120 | 199 | 208 | 182 | July 25 |
| | | | | | | | | | 12 | 40 | 178 | 254 | 192 | 108 | 59 | 65 | 133 | 163 | 111 | Aug. 22 |
| | | | | | | | | | 12 | 40 | 101 | 270 | 85 | 106 | 93 | 78 | 96 | 156 | 92 | Aug. 15 |
| | | | | | | | | | **12** | **40** | 92 | 127 | 78 | 144 | 142 | 106 | 139 | 157 | 180 | Aug. 16 |
| 113 | 14.5 | 3.5 | 59.5 | 108 | 221 | 128 | 128 | 2,546 | 4 | 48 | 96 | 174 | 89 | 146 | 93 | 84 | 93 | 87 | 222 | Aug. 22 |
| | | | | | | | | | **4** | **48** | 101 | 192 | 87 | 124 | 85 | 78 | 88 | 89 | 144 | Aug. 29 |
| 94 | 15.9 | 3.0 | .... | 133 | 235 | 138 | 135 | 2,699 | 4 | 32 | 228 | 190 | 106 | 157 | 102 | 104 | 135 | 165 | 162 | Aug. 9 |
| | | | | | | | | | **4** | **32** | 212 | 230 | 159 | 137 | 116 | 84 | 139 | 186 | 144 | Aug. 15 |

* Mixtures are indicated by the dosage figures in boldface type.

RESULTS

The Table gives data on 11 of the 23 boys. This is an average representation of the whole. In the other 12 subjects the fasting blood-sugar levels were not close enough for comparison of the curves throughout the day. The Table shows the camper's age, duration of diabetes, height and weight on admission to camp, and diet permitted during his camp stay. Also shown are the insulin doses, separate and mixed, for each boy, together with the results of the blood-sugar determinations on the date designated. In certain instances, as in Case 96, the camper was with us a sufficient period to permit procurement of more than one pair of curves.

During the summer, 120 blood-sugar curves were obtained. Half of this group were curves made while separate injections of the insulins were given; the other half were determined while mixed injections were given. Figure 1 is a scattergram of the values obtained after separate injections of the two insulins. One can here view the trend, as well as the extremes, at any period of the day. Also shown is a curve which is the result of the arithmetical averages at each two-hour interval.

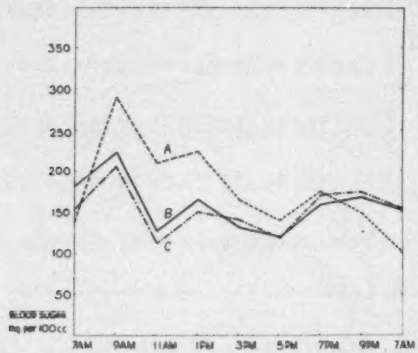


Fig. 3.—Curves showing the average capillary blood-sugar response after injections of crystalline and NPH insulin. *A*, composite curve of 18 tests carried out on 10 campers after the use of NPH insulin alone (Gabriele and Marble[8a]). *B*, average response obtained after injections of mixtures of crystalline and NPH insulins (Fig. 2). *C*, average response obtained after separate injections of comparable doses of crystalline and NPH insulins (Fig. 1). Note the close similarity of curves *B* and *C*.

Figure 2 reveals the same information after injection of a mixture of the two insulins. Figure 3 permits the comparison of two curves which represent the arithmetical averages from Figures 1 and 2. It also shows for comparison a curve of the arithmetical averages of 18 blood-sugar curves obtained from 10 campers (after the use of NPH insulin alone), as reported by Gabriele and Marble.[8a]

Of the 120 blood-sugar curves obtained from the study, i. e., 60 pairs, it was possible to study 37 pairs in some detail because identical insulin doses had been used and starting fasting levels were quite close. In 26 pairs the pattern and results were quite alike. In nine pairs it was noted that mixing of the two insulins permitted higher blood-sugar values by 25 mg. or more at the 9-a. m. peak. In only two of the 37 pairs were higher 9-a. m. peaks noted after the separate injections of the insulins. The incidence of reactions and the degree of glycosuria as measured each 24 hr.

were similar under the two manners of insulin injection. There did not appear to be any significant difference in the results after the combined or separate injection of crystalline and NPH insulins; as the values obtained at any given hour following the two methods of injection were quite comparable, the trend of the blood-sugar levels throughout the day was also similar. In fact, the curves from the arithmetical averages were very close (Fig. 3). Figure 3 also shows how the addition of crystalline insulin may improve the forenoon hyperglycemia which is sometimes present when NPH insulin alone is used. The addition of crystalline insulin tends to produce somewhat lower afternoon blood-sugar values than those that follow use of NPH insulin alone. After supper, however, the slight rise and fall in blood sugar, with maintenance of a good level throughout the night, are totally the effect of the NPH insulin.

## CONCLUSIONS

The results of this study suggest that the giving of a mixture of crystalline (or regular) insulin with NPH insulin produces an effect quite comparable with that obtained when the two insulins are given separately.[7] This is probably due to the fact that the NPH insulin, containing a smaller amount of protamine, does not allow the degree of absorption noted when crystalline insulin is mixed with protamine zinc insulin. Since the mixture of NPH and crystalline insulins in a syringe gives essentially the same results as the injection of the two insulins separately, the simultaneous use of crystalline and NPH insulin in the control of certain diabetic patients becomes more practicable. Such patients are, of course, those in whom the use of NPH insulin alone allows significant hyperglycemia after breakfast.

7. It should be pointed out that when mixtures of crystalline and NPH insulin are made in a syringe the accepted practice is to draw the crystalline insulin into the syringe first. This means that one thereby draws in also the small amount of insulin present in the "dead space" of the tip of the syringe and lumen of the needle. This amounts to about 0.05 cc., which in the case of an insulin concentration of 40 units per cubic centimeter would yield 2 units, and in the case of an insulin concentration of 80 units per cubic centimeter, 4 units. Therefore, the values given for the amount of crystalline insulin in mixtures are slightly larger than stated. Consequently, the doses used in our study with reference to the crystalline type were not entirely identical. Although in view of these facts one must conclude that slightly more rapid action of the crystalline type is lost in a mixture than at first glance might be apparent from our studies, nevertheless in a practical sense the conclusions are valid.

# PNEUMATOSIS CYSTOIDES INTESTINALIS

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WHILE pneumatosis cystoides intestinalis is not a common condition, it occurs frequently enough to warrant the attention of the clinician, because its unique features should readily lead to its recognition. Moreover, while the exact mode of its origin is not clear, the condition seems causally related to other commoner disorders; in such cases, awareness of the existence of pneumatosis cystoides intestinalis may aid in the diagnosis or therapy of the associated disease.

This condition involves the presence of gaseous cysts of varying size within the walls of the stomach and of the intestine. If, as often occurs, some of the cysts rupture, air enters the peritoneal cavity, leading to the clinical and roentgenologic picture of pneumoperitoneum, which may prompt the erroneous diagnosis of perforation of a viscus. The roentgenographic appearance of gaseous cysts, with or without pneumoperitoneum, is so characteristic that the correct diagnosis should not be missed.

## HISTORICAL REVIEW

Many reports deal only with single cases; the number of articles concerning this condition is considerable. There are, however, reports[1] which summarize the observations of other authors and present a fair picture of the clinical, roentgenologic, and pathological features of pneumatosis cystoides intestinalis and discuss its pathogenesis.

According to most authors, the disorder was described for the first time in 1876 by Bang.[2] However, a much earlier report on an observation made by Johann G. Du Vernoi (also known as Duvernoy) was published in the Commentaries of the Imperial Academy of Science of Petersburg for the years 1730-1731 and printed in 1738.[3] This paper includes a lucid and complete description of a case observed

From the Diagnostic X-Ray Department, Medical Department "A," and Anatomo-Pathological Department of the Hadassah University Hospital and Medical School.

1. (*a*) Stoyanovitch, M. D.: Kystes gazeux de l'intestin, Thèses de la Faculté de Médecine de Paris, 1919. (*b*) Mills, H. W.: Gas Cysts of the Intestine, Surg., Gynec. & Obst. **40**:387, 1925. (*c*) Ferrandu, S.: Studio anatomo-patologicae interpretazione patogenetica della pneumatosi intestinale cistica, Arch. sc. med. **60**:493, 1935. (*d*) Dressler, M.: Über die Pneumatosis cystoides intestini, Helvet. med. acta **6**:229, 1939. (*e*) Vaz, O.: Pneumatose cística intestinal, Rev. méd. munic. **2**:449, 1941.

2. Bang, B. L. F.: Luftholdige kyster i väggen af ileum og i nydannet bindevāv på sammes serosa, Nord. med. ark. (No. 18) **8**:1-16, 1876.

3. Du Vernoi, J. G.: Aer intestinorum tam sub extima quam intima tunica inclusus: Observationes anatomicae, Comment. Acad. scient. imp. Petropol. 1730, Vol. 5, p. 213.

at autopsy with all the major pathological findings which later became accepted as characteristic of the disease and contains a discussion of the possible mode of origin of this condition. The anatomical description of this case is quoted in full in a book by F. de Paula Combalusier, printed in 1747.[4] Another case was reported by Cloquet in 1820,[5] and although the true character of the gaseous cysts observed in this case was questioned by some later authors, a perusal of the original report leaves no doubt that it deals with lesions similar to those which were later described under the name pneumatosis cystoides intestinalis. Cloquet had previously seen and described similar lesions in swine and mentioned them again in his report on the human case. Bang's case, published in 1876, seems to be the third case on record, and it is worth mentioning that he cites the two cases referred to above. His is, however, the first case report accompanied with illustrations showing both the gross appearance and the microscopic structure of the gaseous cysts.

The first in-vivo observation of this condition in the course of an operation was reported by Hahn in 1899,[6] and thereafter it was repeatedly seen during surgical operations and postmortem examination. In American medical literature the condition is usually called "gas cysts of the intestine," and it was under this title that the first American case was published by Finney in 1908.[7] In 1925 Mills [1b] summarized 100 cases and listed more than 120 papers dealing with the subject; in a thorough study by Vaz in 1941 [1c] the total count included 190 cases. As additional cases were published during the last decade, it seems probable that today about 200 cases are known.

A condition similar to that seen in man has been described not infrequently in swine. It was observed in 1818 by Cloquet,[5] and a complete description and discussion were given by Mayer in 1825.[8] In his paper there appears for the first time the name "pneumatosis cystoides intestinorum," which was adopted by many of the later observers of human cases. It is of further interest that in Mayer's case the first analysis of the gas contained in the cysts was carried out. A similar condition has been observed, although much less frequently, in sheep and in chicks, and it is important to note that the disorder has been created in swine experimentally, a fact which will be discussed in connection with the possible cause of its occurrence in patients.

Pneumatosis cystoides intestinalis assumed increased interest when it became apparent that this condition presents pathognomonic roentgenographic signs and can be diagnosed by this method. While the roentgenologic appearance was described by Barjon and Dupasquier in 1913,[9] and subsequently by Moreau [10] and other authors, as definitely related to the operative findings of gas cysts within

4. Combalusier, F. de P.: Pneumato-pathologia seu tractatus de flatulentis humani corporis affectibus, Paris, 1747, p. 17 ff.

5. Cloquet, J.: Emphyseme du tissu cellulaire qui réunit les tuniques de l'estomac, Bull. Fac. Soc. de Méd. Paris **8**:267, 1820.

6. Hahn, E.: Über Pneumatosis cystoides intestinorum hominis und einen durch Laparotomie behandelten Fall, Deutsche med. Wchnschr. **25**:657, 1899.

7. Finney, J. M. T.: Gas Cysts of the Intestine, J. A. M. A. **51**:1291 (Oct. 17) 1908.

8. Mayer, A. F.: Beobachtung einer Pneumatosis cystoides intestinorum, J. prakt. Heilk. **61**(Pt. 2):67, 1825.

9. Barjon, F., and Dupasquier, D.: Kystes gazeux de l'intestin, Lyon. méd. **121**:565, 1913.

10. Moreau, L.: Sur un cas d'hépatoptose par interposition avec kystes gazeux de l'intestin: examination radiologique vérifié par l'intervention, Arch. d'élec. méd. physioth. **25**:393, 1917.

the abdominal cavity, the number of cases in which the condition was actually diagnosed preoperatively is still very small. The first case on record (1924) is that of Reverdin,[11] who was able, on the basis of his experience with a previous patient, to arrive at a diagnosis of the disease which was confirmed by the operative findings. Other authors [12] discuss the correlation between the roentgen signs and the anatomical conditions adequately, but most of them admit that this knowledge was arrived at only after surgical detection of the condition. The first roentgenologic diagnosis in America was made in 1946 by Lerner and Gazin,[13] lacking, however, operative proof. But the same authors were able to publish another case in 1949 [14] also diagnosed by means of the roentgenogram, with later confirmation by the operative findings.

## CLINICAL FINDINGS

It is impossible to attribute a clear-cut clinical picture to the presence of multiple gaseous cysts within the walls of the digestive tract, and most of the patients' complaints seem unrelated to this condition. It seems, however, that in a large majority of the reported cases the underlying condition presenting symptoms was a stenosing lesion, usually an ulcer, in the pyloric region of the stomach and occasionally an obstruction at a lower point along the gastrointestinal tract, due to adhesions, volvulus, or similar conditions. This coincidence of stenosing conditions and the presence of gas cysts is frequent enough to point to a possible causal relationship between them. On the other hand, the gas cysts themselves may narrow the lumen of the intestine to an appreciable degree and thus bring about a partial or complete obstruction with its clinical manifestations.

In many cases, superficial gas cysts rupture and air accumulates in the peritoneal cavity. Usually the presence of air, except if in large amounts, will not cause noticeable clinical signs. It can, however, be readily detected on physical examination and by roentgenologic examination.

## ROENTGENOLOGIC SIGNS

One feature of pneumatosis cystoides intestinalis which is frequently, but not always, present is pneumoperitoneum. Presence of free air in the peritoneal cavity per se is not, of course, evidence of a specific pathological condition, as such air may arise from a variety of disturbances. If, however, subdiaphragmatic air is observed over a prolonged period, and if perforation of a viscus can be excluded

11. Reverdin, A.: Maladie kystique de l' intestin (Pneumatosin intestinalis), Rev. méd. Suisse Rom. **44**:545, 1924.

12. (*a*) Ruckensteiner, E., and Kux, E.: Über die Pneumatosis cystoides intestini und die Möglichkeit ihrer röntgenologischen Diagnose, Fortschr. Geb. Röntgenstrahlen **47**:661, 1933. (*b*) Gråberger, G.: Beitrag zur Kenntnis des Röntgenbildes bei Pneumatosis cystoides intestinorum, Acta radiol. **16**:439, 1935. (*c*) Stenström, B.: Reflexionen über die Ätiologie des spontanen Pneumoperitoneums gelegentlich eines Röntgenbefundes, ibid. **18**:625, 1937. (*d*) Baumann-Schenker, R.: Über Pneumatosis cystoides ventriculi et jejuni, ibid. **20**:365, 1939. (*e*) Berglund, S.: Roentgen Diagnosis of Pneumatosis Cystoides Intestinorum Hominis, ibid. **20**:401, 1939. (*f*) Frostberg, N.: Pneumatosis cystoides intestini, ibid. **29**:521, 1948.

13. Lerner, H. H., and Gazin, A. I.: Pneumatosis Intestinalis: Its Roentgenologic Diagnosis, Am. J. Roentgenol. **56**:464, 1946.

14. Gazin, A. I.; Brooke, W. S.; Lerner, H. H., and Price, P. B.: Pneumatosis Intestinalis, Am. J. Surg. **77**:563, 1949.

on clinical evidence, pneumatosis cystoides intestinalis should be considered because the persistent formation and rupture of gas cysts is the only known condition which can readily explain a prolonged or recurrent pneumoperitoneum.

In a fair number of cases of this disease, the presence of loops of small intestine between the liver and the diaphragm has been observed on roentgenologic studies. In these cases, too, there exists an abnormal translucency above the liver; but, in contrast to the picture of pneumoperitoneum, this area of high translucency is not uniform but shows archlike lines delineating the intestinal loops. Moreover, this translucency does not shift, as free air does, when the patient is examined in different positions. This interposition of small intestine was first described by Chilaiditi in 1911 [15] without relation to pneumatosis cystoides intestinalis and later observed in this condition by Moreau in 1913.[10] According to Ruckensteiner and Kux,[12a] the air cysts within the walls of the small intestine make it buoyant and enable it to enter the space between liver and diaphragm.

The gas cysts themselves are in many cases, as also in our case, clearly visible because of their foamlike appearance wherever they are present and are to be regarded as the most certain roentgenologic evidence of pneumatosis cystoides intestinalis.

On examination of the small intestine with barium sulfate suspension, one can occasionally observe that the lumen is narrowed by the pressure of the small submucosal air cysts, while the outline of the small intestine becomes irregular by a chain of small filling defects.

## PATHOLOGICAL ASPECTS

Gas cysts under the serous covering of the intestine present a striking and often bizarre picture. The cysts may vary in size from pinheads to 1 in. (2.5 cm.) or more in diameter; they may occur singly or in clusters. They may cover the intestine, even surrounding it in part with a layer of foamlike structure, or they may be attached to it by pedicles and spread out in grapelike fashion. When cut into, the larger accumulations appear like hydatid moles or, as stated by Duvernoy, like a honeycomb. On the inner aspect of the intestine the cysts are visible lying below the mucosal lining and elevating it, sometimes to such a degree as to narrow the lumen appreciably. As in other cases of tissue emphysema, the air may spread from its original site to distant parts, and in cases of pneumatosis cystoides intestinalis it has been found in the mesentery (apparently in the lymph channels and nodes), in the mediastinum,[16] between the muscular layer and the peritoneal covering of the diaphragm under the parietal peritoneum, and in other places.

The cysts contain no fluid but only air, which is described unanimously as lacking odor and by most authors as nonburning. Its composition seems similar to that of atmospheric air, there being, however, vast differences in the various reports on its analysis. It is conceivable that these differences might reflect different stages of selective reabsorption of the various components of the gas mixture within the cysts.

15. Chilaiditi, D.: Zwei Fälle von temporärer partieller Leberverlagerung, Beibl. Mitt. Gesellsch. inn. Med. Kinderh. **9**:74, 1911.

16. Fauchamps, A.: Une localisation nouvelle de la lymphopneumatose kystique: lymphopneumatose du médiastin, Schweiz. Ztschr. Path. & Bakt. **8**:208, 1945.

The histologic picture of the tissues containing the gas cysts has been widely studied and described. The most important aspect of these studies lies in the fact that the air spaces correspond usually, but not invariably, to lymph channels. The cysts are typically lined with a layer of flat endothelial cells, resembling closely those of the lymph channels. The walls surrounding the cysts and separating them from each other consist of sparse connective tissue, with some elastic fibers. Many of the cysts show the endothelial lining not at all, or only partially. This may be explained by the assumption that the original cysts can rupture into the surrounding tissue, as they are known to do into the peritoneal cavity, and thus form new, less well-organized air spaces. In many places within the endothelial lining and interstitial tissue, giant cells containing several nuclei have been found, equivalent to the foreign-body giant cells seen in many other conditions. Not infrequently, one finds signs of an inflammatory reaction in the interstitial tissue, consisting of histiocytes and hemorrhages. Comparison of various lesions in the same case leads to the conclusion that a well-defined development is taking place, leading finally to scar tissue. The scars may appear on the serosa as small whitish nodules, which some observers have seen after the cysts themselves have disappeared.

### ETIOLOGICAL FACTORS

It is still not clear how the gas cysts are formed, and various explanations have been put forward. If we discard as entirely unsubstantiated the older "neoplastic" and "infectious" theories, according to which the air originates either from special "gas-forming" tumor cells or from an unidentified bacterium, we find the "mechanical" theory as the most frequently discussed. According to this theory, the obstruction which is present in most of the cases, together with a small break in the continuity of the mucosal layer of the intestine, causes air to be forced from the lumen of the bowel into its wall. The difficulties in this explanation are apparent in that the vast majority of patients with intestinal obstruction exhibit no such lesions; in fact, in most cases of pneumatosis cystoides intestinalis the cysts are located over a wide area, far below the seat of the obstruction, making it unlikely that they all result from one initial break in the continuity of the mucosal wall. One other possible factor in the genesis of the gas cysts come to the fore through the animal experiments which have been mentioned briefly. Eveleth and Biester [17] have shown that in hogs fed on polished rice gas cysts covering the serosa of the intestine can be brought about. In discussing these experiments, Friedlaender and Teitelbaum [18] suggest that in human cases there may also be a nutritional factor, probably on the basis of avitaminosis, active either alone or in combination with other factors. We were impressed by the rather frequent mention of the descriptions "bad nutritional state" or "severe weight loss" in the clinical histories of the reported cases and should like to suggest that these patients may really have been suffering from avitaminotic conditions in connection with their obstructive lesions in the digestive tract. In our case there was little doubt on this point. In other cases in which there was no obstruction, other wasting diseases, such as tuberculosis of the intestine, were mentioned.

17. Eveleth, D. F., and Biester, H. E.: Further Studies on Intestinal Emphysema of Swine, Am. J. Hyg. **27**:364, 1938.

18. Friedlaender, S. O., and Teitelbaum, S. S.: Gas Cysts of the Intestines: Cystic Pneumatosis, West. J. Surg. **58**:192, 1950.

REPORT OF A CASE

The patient, a white man aged 70 yr., was first admitted to the Hadassah University Hospital in February, 1945. He had been suffering from pain in the abdomen for about 25 yr., had made frequent use of vermifuges, and had often had periods of vomiting. During the preceding two years, he had experienced hunger pain which was relieved by small feedings, since large amounts of food caused vomiting. He had actually become afraid to eat and had progressively reduced his food intake. The episode which brought him to the hospital was the occurrence of melena a week before he was admitted; this was the second such episode in three months.

On physical examination the patient showed signs of extreme malnutrition. His weight was only 38 kg.; his skin was dry and atrophic, with follicular keratitis, and pellagra in the form of an acute dermatitis was present on the hands and forearms. The tongue was smooth and atrophic and appeared very red. Teeth were lacking. The patient had dry rales over both lungs; otherwise the physical examination of the thorax did not reveal any abnormality. The liver could be felt about 2 fingerbreadths below the costal margin; it was firm, not tender. The entire abdomen appeared somewhat protruding, and meteorism was noted. Rectal and neurological examination showed normal results. Laboratory examinations revealed 6,000,000 red blood

Fig. 1.—Roentgenogram in left decubitus position showing pneumoperitoneum. Note two shadows of calcified Echinococcus cysts on the liver.

cells and 8,400 white blood cells per cubic millimeter, with a normal differential count. The urine showed reactions for albumin on two examinations; Ascaris eggs were observed in the feces. The stomach contents were highly acid (free acid 70 and total acidity 100 clinical units).

At this time roentgen examination revealed pyloric stenosis; the duodenal bulb could not be visualized. Within the liver two round shadows were visible and interpreted as calcified Echinococcus cysts. Subsequently, Weinberg and Casoni tests were found to give positive reactions for Echinococcus.

After an uneventful interval of approximately one and one-half years, the patient was readmitted on July 14, 1946, with similar complaints. His general condition, too, was essentially unchanged. On fluoroscopic examination, however, a large amount of air was noted under the diaphragm on both sides. Recheck of his history did not give any indication of a perforation in the recent past. Plain roentgenograms taken at this time showed interposition of the small intestine between the liver and the diaphragm; foam-structured, multiple clusters of cystlike appearance were seen in the upper parts of the abdomen (Figs. 1 and 2). Examination of the small bowel with barium sulfate suspension showed multiple, irregular narrowings in the course of the intestine (Fig. 3). Two months later the pneumoperitoneum was still present, and at that time the diagnosis of "pneumatosis cystoides intestini" or "gas cysts of the intestine" was

Fig. 2.—Roentgenogram in upright position, showing interposition of small intestine between liver and diaphragm.

Fig. 3.—Bucky prone roentgenogram, showing foam-structured, multiple cystlike clusters, especially in the left side of the abdomen.

made. The patient at all times declined any surgical treatment for the pyloric obstruction and received only moderate relief from medical therapy applied during his repeated stays in our hospital and his visits to the outpatient department. Roentgenographic studies were repeated on various occasions and always showed a picture similar to that described above.

Fig. 4.—Roentgenogram taken six hours after barium sulfate meal, showing pyloric stenosis with marked enlargement of the stomach. Note the irregular outlines with multiple filling defects and cystlike indentations of various loops of the small intestine.

Fig. 5.—Cysts on intestinal wall.

On Oct. 23, 1949, he was brought to the hospital in a much worse condition. He was at that time about 75 yr. old, had been vomiting all his food during the past two weeks, and was cachectic from undernutrition. Parenteral fluid therapy was instituted, but the patient died after four days.

*Autopsy Observations.*—Autopsy was performed three hours after death. The pertinent findings were as follows.

External Examination: The body was that of an undernourished man about 70 yr. old. No postmortem rigidity was present. Marked edema was noted in the left leg and slight edema in the right. The skin of the dorsal aspect of both hands was dry and contained a few petechial hemorrhages. No evidence of jaundice could be detected.

Internal Examination: Thorax: One hundred to 150 cc. of clear, yellowish fluid was found in each of the pleural cavities. Fibrous adhesions, easily torn, were present between the pleurae.

Peritoneal cavity: The peritoneal cavity contained 150 cc. of clear, yellowish fluid. The stomach was greatly distended. A loop of the jejunum adhered to the anterior aspect of the left lobe of the liver and to the falciform ligament.

Fig. 6.—View of intestine with cysts.

Stomach: The stomach contained a large amount of brown-yellowish fluid. Four ulcers were seen in the mucous membrane. One of them was situated in the middle portion of the lesser curvature, and the other three were in the prepyloric region. The tissue surrounding the ulcers was firm. The ulcers were deep, and one of them almost reached the serous membrane. The base of the ulcers was partly covered by amorphous material; the margins were not undermined. The pylorus was greatly constricted.

Small intestine (Figs. 5 and 6): All along the serous surface of the small intestine cysts were observed. The cysts either formed groups or were solitary. Their walls were transparent, and no other contents but gas could be found in them. No direct communication was observed between the lumens of the different cysts. Their cut surfaces revealed smooth and transparent walls. In many places the cysts almost reached the mucous membrane and caused it to bulge (Fig. 7). The mucous membrane and the folds did not reveal any pathological changes.

Colon: No cystic formations were observed in the colon, and no pathological changes were detected in the mucous membrane or the intestinal wall.

Liver: The weight of the liver was 1,800 gm. Glisson's capsule was smooth and glistening, except for an area in the anterior surface of the left lobe, where it was thickened because of the adhesions described above. Inside the liver two Echinococcus cysts were seen, partially calcified.

*Microscopic Examination.*—Six pieces of tissue taken from different sites on the small intestine were examined. The sections were stained with hematoxylin and eosin, the Van Gieson, the Weigert elastic, and the Petersen stains. The histological picture was similar in all the sections examined.

The mucous membrane was well preserved, containing scattered polymorphonuclear granulocytes and congested blood vessels. In the submucosa and muscularis, there were several

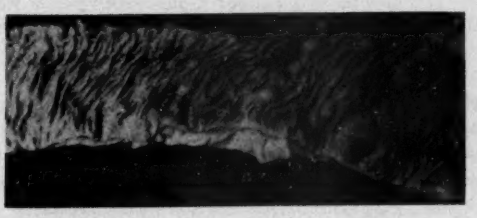

Fig. 7.—Bulging of mucous membrane caused by cysts.

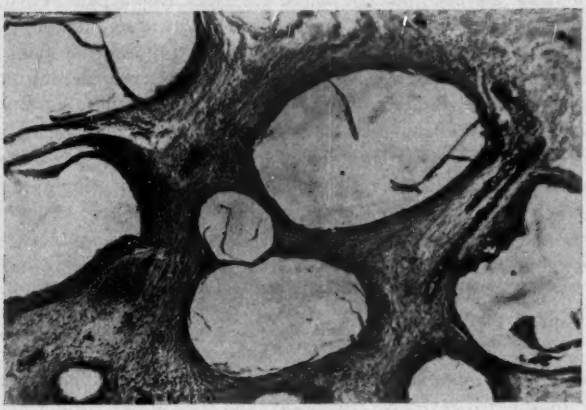

Fig. 8.—Photomicrograph of cysts.

empty cystlike spaces of different sizes and shapes. In some areas, particularly in those corresponding to the cystic spaces, the mucous membrane bulged into the intestinal lumen and was atrophied. The cysts were demarcated by a connective-tissue wall, neatly defined in some cysts while ill-defined in others (Fig. 8). The connective-tissue walls varied in regard to their cellularity. In some of them, among the collagenous fibers, there were fine or coarse elastic fibers. The lumens were lined by a single, though not continuous, layer of cells with a narrow and elongated, or swollen and vesicular, nucleus. Here and there, in the neighborhood of the above-mentioned lining layer, several giant cells of the foreign-body type were observed. Many of these giant cells lined the walls of the cysts; others protruded into the lumen, while some of them were seen lying in the lumen with no apparent connection to the wall. The cysts caused

a complete deformation of the normal architecture of the intestinal wall. The intestinal layers contained, among the cysts, scattered lymphocytic infiltration.

In the material examined there were various forms of cysts which are believed to represent different stages in the process of healing and organization. Some of these forms were represented by a narrow lumen surrounded by a thick fibrous, partly hyaline, ring. Other forms were represented by fibrotic nodules without any lumen.

*Anatomical Diagnoses.*—Anatomical diagnoses were as follows: (1) pneumatosis of small intestine; (2) pyloric stenosis; three chronic prepyloric ulcers; one peptic ulcer of small curvature of stomach; (3) chronic perihepatitis; fibrous adhesions with intestinal loops of jejunum; (4) marked edema of legs; (5) ascites, about 150 cc. of fluid; (6) bilateral hydrothorax (150 cc.); (7) bilateral bronchiectasis, with bronchiectatic abscesses in apex of right lung and lower lobes of both lungs; purulent bronchitis; (8) bilateral fibrous adhesive pleurisy; (9) arteriosclerosis; (10) two partially calcified Echinococcus cysts of right upper lobe of liver; (11) fibrous perisplenitis; (12) foci of bronchopneumonia in base of left lung.

## COMMENT

This case, which in general closely resembles those previously described, deserves special attention for a number of reasons. While in most other cases pneumatosis cystoides intestinalis has been observed only for a short period, in our case the condition could be demonstrated and closely followed for about four years by repeated roentgenologic examinations and was finally proved by autopsy. It can be assumed that the persistence of the underlying causal condition may sustain the existence, or cause the re-formation, of the gas cysts over a long period. While usually such a condition is corrected by surgical treatment as soon as it is recognized, in our case it was present in an unabated form during the long period of continued observation. In addition, we believe that the severe undernutrition which was also present for the duration of our observations, and which led to a state of avitaminosis, was a further cause for the persistence or recurrence of the gas cysts. Neither factor alone has so far been shown to cause this strange condition, apart from the similar condition in swine which could be produced by feeding experiments. In this case their combination for a prolonged period may be regarded as the main reason for the pneumatosis cystoides intestinalis. It must be remembered, however, that even with this assumption we are not in a position to explain the actual mechanism by which the gas is extruded from the intestinal lumen or formed within the walls of the bowels.

## SUMMARY

Knowledge of the condition known as pneumatosis cystoides intestinalis or gas cysts of the intestines is reviewed historically, and the clinical, roentgenologic, and pathological features of this condition are described.

A case is reported in which this condition was diagnosed on roentgen examination, observed for about four years, and finally proved by autopsy.

A persistent accumulation of subdiaphragmatic air, seemingly without symptoms, is considered by us to be a pathognomonic sign of pneumatosis cystoides intestinalis.

On the basis of other cases in the literature and the case presented here, the combination of intestinal obstruction and severe malnutrition, with avitaminosis, is discussed as a prerequisite for the occurrence of this condition.

# NONCLOSTRIDIAL GAS INFECTION IN THE DIABETIC

## Review of the Literature and Report of Three Cases

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ALL OF us are familiar with gas gangrene, the infection produced by Clostridium perfringens (Bacillus welchii). However, very few of us know that other organisms, such as Escherichia coli and anaerobic Streptococcus, can also produce gas infection in tissues.

In 1825 Boismont [1] first mentioned the spontaneous production of gas in the urinary bladder. He considered it to be a secretion by the mucosa. Chomel [2] added the suggestion that the gas might be due to fermentation. Guiard [3] first pointed out the occurrence of pneumaturia in diabetic persons in 1883 and reported four cases. In 1888 Favre [4] isolated a gas-forming bacterium from the urine of a nondiabetic person who died. Chiari [5] in 1893 was the first to describe the formation of gas due to the colon bacillus in the gangrenous tissues of the leg of a diabetic. Animal inoculation with the organism failed to produce gas. He believed that the diabetic state favored the production of gas in the soft tissues. Hitschmann and Linderthal [6] in 1899 reported a similar case. Their patient was a diabetic who had a foot infection. Clinically he was thought to have gas gangrene, but bacteriologically E. coli, Streptococcus pyogenes, and an unidentifiable rod were the organisms isolated. Of these, E. coli was the only organism that produced gas on culture. Marwedel and Wehrsig [7] in 1915 reported two cases of gas infection in nondiabetic patients from whom the anaerobic Streptococcus was isolated. Warren [8] in 1938

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Read Before the Meeting of the Clinical Society of The New York Diabetic Association at the New York Academy of Medicine, New York, Feb. 8, 1951.

1. Briene de Boismont, cited by Kelly, H. A., and MacCallum, W. G.: Pneumaturia, J. A. M. A. **31**:375 (Aug. 20) 1898; cited by Riley, F. G., and Bragdon, F. H.: Pneumaturia in Diabetes Mellitus, ibid. **108**:1596 (May 8) 1937.

2. Chomel, cited by Riley, F. G., and Bragdon, F. H.: Pneumaturia in Diabetes Mellitus, J. A. M. A. **108**:1596 (May 8) 1937.

3. Guiard, F. P.: Ann. mal. org. génito-urin. **1**:242, 285, and 363, 1883.

4. Favre, A., cited by Riley, F. G., and Bragdon, F. H.: Pneumaturia in Diabetes Mellitus, J. A. M. A. **108**:1596 (May 8) 1937.

5. Chiari, H.: Prag. med. Wchnschr. **18**:1, 1893.

6. Hitschmann, F., and Linderthal, O.: Sitzungsbericht kaiserl. Akad. Wissensch. Math.-naturw. Cl. **108**:145, 1899.

7. Marwedel and Wehrsig: München. med. Wschnschr. **62**:1023, 1915.

8. Warren, S.: The Pathology of Diabetes Mellitus, ed. 2, Philadelphia, Lea & Febiger, 1938, p. 160.

mentioned two cases of diabetics in whom a clinical diagnosis of gas gangrene was made, but repeated cultures, both aerobic and anaerobic, failed to reveal anything but the presence of E. coli and Staphylococcus. In a third case, that of a diabetic with a perinephric abscess, the formation of gas outlined the kidney strikingly in the roentgenogram. Gillies [9] in 1941 described four cases of diabetics with infection due to the colon bacillus alone or in association with other organisms. One patient had gas formation perirenally, and three had it in the lower extremities. The diabetic state is not necessary for the production of gas in tissues by E. coli. However, the increased dextrose content of the body fluids and tissues in the diabetic offers a more favorable situation for the production of gas. The reported cases [10] of the formation of gas in the urinary tract of the diabetic with colon-bacillus infection support this thesis. Altemeier and Culbertson [11] in 1948 reviewed the literature from the surgical viewpoint and analyzed 12 cases of their own. One of these was of a diabetic who had a foot infection. Culture of the pus showed the presence of Staphylococcus, Streptococcus, and bacilli, but no colon bacilli. In Altemeier's [12] experience, such infections are usually not controlled by antibiotic therapy alone.

In 1929 Reis [13] reported an interesting case in which gas thought to be due to E. coli was found in the eye of a diabetic patient.

We have been led to present this paper because this unusual condition is generally confused with gas gangrene; its early differentiation from that condition yields a different problem in therapy and prognosis. Recently, one of us (M. S.) had occasion to see a diabetic man who acquired a nonclostridial gas infection. A review of the records of The Bronx Hospital from 1932 to 1950 revealed two more such cases. These three cases are described in detail.

## REPORT OF CASES

Case 1.—P. D. (228951), a 46-yr.-old white man, was admitted to the private medical service of one of us (M. S.) at 5 p. m. on Aug. 14, 1950, in diabetic coma. He was a known diabetic for 19 yr. For the past seven years he had not seen a physician. He was on a diet and received 55 units of protamine zinc insulin daily. The diabetes was poorly controlled on this regimen. Two days before admission he began to vomit and did not eat. Progression to the state of coma resulted. On examination he was noted to be in coma and to have Kussmaul breathing. The temperature was 98.6 F. The pulse rate was 114 beats a minute, and respirations were 26 a minute. The blood pressure was 150/70. The eyeballs were soft. The knee and ankle jerks were absent, and no Babinski sign was present. Results of the remainder of the examination were noncontributory.

*Laboratory Studies.*—The hematocrit was 33 cc. per 100 cc. of blood. Urinalysis revealed a trace reaction for albumin, a 4+ reaction for sugar, a 1+ reaction for acetone, and no reaction for diacetic acid. Microscopically there was a rare white blood cell. Blood sugar was 400 mg. per 100 cc., and the carbon dioxide-combining power was 18 vol. %. The red blood cells numbered 3,620,000, with 72% (10.6 gm.) hemoglobin (Sahli). There were 12,200 white blood cells, with 82 polymorphonuclear forms, 17 lymphocytes, and 1 monocyte on differential smear.

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9. Gillies, C. L.: Interstitial Emphysema in Diabetes Mellitus Due to Colon Bacillus Infection: Report of 4 Cases, J. A. M. A. **117**:2240 (Dec. 27) 1941.

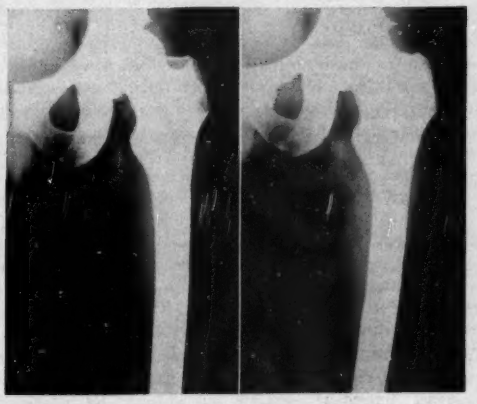
10. Marquardt, C. R.: Urol. & Cutan. Rev. **44**:295, 1940. Olsonn, O. G.: Acta radiol. **20**:578, 1939. Sanes, S., and Doroshow, G.: J. Urol. **32**:278, 1934.

11. Altemeier, W. A., and Culbertson, W. R.: Surg., Gynec. & Obst. **87**:206, 1948.

12. Altemeier, W. A., in Monographs on Surgery, 1950, edited by B. N. Carter, New York, Thos. Nelson & Sons, 1949, pp. 59-60.

13. Reis, W.: Klin. Monatsbl. Augenh. **83**:784, 1929.

*Course.*—The patient was treated vigorously with oxygen and dextrose in saline solution given intravenously, alternating with isotonic sodium chloride solution and M/6 lactate solution plus 50 units of insulin every one-half hour. A Foley retention catheter was inserted into the bladder. Within 14 hr., and after 1,250 units of insulin had been given, acetone had disappeared from the urine, but the patient was still comatose and had Cheyne-Stokes respiration. A spinal tap revealed a high pressure, but there was no evidence of intracerebral hemorrhage. Dr. George Brown saw the patient in consultation and suggested the use of potassium chloride intravenously, though there was no evidence of hypopotassemia. Six grams was given, starting at 4 p. m. on Aug. 15. By this time, 22 hr. after admission, the urine had become sugar-free and 1,415 units of insulin had been given. On Aug. 16, 40 hr. after admission, the patient was responsive to questions, though confused. Breathing was normal. Sixty-four hours after admission, there was normal cerebration and the patient was out of coma. After removal of the Foley catheter, the urine was found to be purulent and the temperature rose to 104 F. Culture of the urine revealed the presence of Proteus vulgaris (Bacillus proteus). Treatment with aureomycin and gantrisin® (3, 4-dimethyl-5-sulfanilamido-isoxazole) was started. On Aug. 19, four days after a clysis of M/6 lactate solution had been given in the left



Roentgenograms of the left thigh in Case 1: *A*, anteroposterior view; *B*, lateral view. Note gas in tissues of lateral aspect of thigh.

thigh, the patient complained of pain at the site of the clysis. The muscle was found to be tender in this area. Heat appeared within three days, but no swelling. (All this time the patient was receiving gantrisin,® aquacillin® [aqueous suspension of penicillin G procaine], and aureomycin.) On Aug. 24, brawny edema became evident in the thigh. Four days later, brawny edema was present in the entire quadriceps muscle and crepitus was felt for the first time. The patient was thought to have a gas-gangrene infection and gas-gangrene antitoxin was given. However, on the 10th day after the onset of the infection, a thin serous material was aspirated from the infected area. Direct smear of this material was negative, but E. coli was grown on the culture. (This organism was observed to be highly sensitive to chloramphenicol (chloromycetin®) and moderately so to aureomycin.) Gas was present in the soft tissues of the thigh on roentgenogram (Fig.). The fever was low grade, the temperature being about 100 F. from the fourth hospital day. On the twelfth day of the infection, Aug. 31, when in spite of antibiotic therapy no progress was being made in controlling the infection, immediate operation was advised. One of us (S. K.) saw the patient in consultation and discovered a deep subcutaneous abscess on the anterior and lateral aspects of the left thigh. There were fluctuation, crepitation, and cellulitis from the greater trochanter to the lateral condyle. The advisability of immediate operation was concurred with. With the patient under general anesthesia, multiple incisions were made in the thigh. Abundant gas and a thin,

malodorous, seropurulent fluid were evacuated. The tissues were grayish-black and edematous. E. coli was cultured from the pus. Postoperatively, chloramphenicol therapy was instituted. Four days after the operation the temperature became normal. The remainder of the course was uneventful. The patient was discharged on the seventh postoperative day and the 25th hospital day.

CASE 2.—W. H. (40952), a 49-yr.-old white male diabetic, was admitted to the private surgical service of Dr. Sidney Cohn, on Sept. 18, 1933, with a typical history of acute appendicitis of 24 hours' duration. The laboratory findings on admission were 139.9 mg. of blood sugar, 15.8 mg. of urea nitrogen, and 3.0 mg. of uric acid, per 100 cc. Urinary sugar was 2.5%, and the reaction for acetone, 1+. There were 20,000 white blood cells, with 88 polymorphonuclear forms and 12 lymphocytes on differential smear. An acutely inflamed, gangrenous appendix was removed by Dr. Cohn that same day. Two weeks postoperatively the patient exhibited crepitation of the abdominal wall. This began around the site of the operative wound and progressed along the fascial planes to the right thigh and leg. Open drainage by means of multiple incisions was instituted in order to free the tissues of gas and pus. E. coli was observed on smear and culture. The patient was discharged cured on Nov. 29, 1933.

CASE 3.—J. R. (116588), a 64-yr.-old white man, was admitted to the private service of Dr. M. F. Levinson on Nov. 6, 1940, because of continuous rectal pain for 10 days, which was aggravated by defecation. Two days before admission an inflamed hemorrhoid was found. The night before admission the patient became aware of fulness and swelling in the anal region. On the day of hospital admission a brawny induration of the entire perianal area extending toward the scrotum was present. In this indurated area there was a small streak of gray-black discoloration. The patient suffered from bronchitis and emphysema.

On physical examination, the temperature was 98.8 F. and the blood pressure was 120/88. Occasional wheezes and sonorous rales were heard over the lung fields. There were tenderness, swelling, purulent discharge, and blue-black discoloration of the anal region, penis, and scrotal sac.

Laboratory studies showed 5,300,000 red blood cells, 104% (15.0 gm.) hemoglobin (Sahli), and 25,000 white blood cells, with 68 polymorphonuclear forms, 25 band forms, 3 lymphocytes, and 4 monocytes on differential smear. The urine had a specific gravity of 1.020; no albumin was detected; sugar was 3%. A 2+ reaction for acetone was present, and no abnormal constituents were seen on microscopic examination.

*Course.*—Dr. F. Frankfeldt saw the patient in consultation. He felt crepitation in the involved area. It was his impression that the patient had diabetes with either a fulminating infection or gas gangrene. Immediate operation was advised. Dr. Frankfeldt did an incision and drainage of a rectal abscess. To quote from his operative report: "Dark-colored blood with a very considerable amount of gas exuded from the wound. The odor was that of putrescence. The tissue exposed was gray-black. The inflammatory process was not deep and seemed to involve only the area immediately below the integument. On palpation one could feel distinct crepitation under the perianal scrotal area." Immediately after the operation, the patient was given deep x-ray therapy, and treatment with sulfanilamide was started. Smear of the evacuated pus showed the presence of Gram-positive cocci in short chains and Gram-positive and Gram-negative bacilli. On culture there was no anaerobic or aerobic growth. Mouse inoculation revealed no Cl. perfringens.

The diabetic state was difficult to control. The following noon the patient was in semicoma and by late afternoon in coma. The blood sugar was 363.3 mg. per 100 cc. at this time. Concomitant with the deepening of the diabetic state, the infection became worse and spread. By 7:30 a. m. the following day the indurated area had spread to below the umbilicus. Crepitus was felt over all the indurated area. The patient was given gas-bacillus antitoxin without avail. At 6:30 p. m. on Nov. 8, two days after admission, he died.

## COMMENT

Previously the presence of crepitus in infected tissue led one to believe that gas gangrene was present. When found in the lower extremity, amputation as a life-saving procedure was resorted to in order to prevent the spread of infection,

especially when arteriosclerosis obliterans was present. However, other organisms, such as E. coli, are capable of producing gas in tissues, especially when an adequate culture medium is present. A medium of this type is found in the high dextrose content of the tissues of the diabetic. In addition, gas may accumulate because the circulation in the diabetic limb is too poor to carry the gas away.

The nonclostridial organisms that produce gas infection may be aerobic or anaerobic. The aerobic include the coliform bacilli (especially the E. coli) and the pyogenic cocci (especially the nonhemolytic Streptococcus), and the anaerobic include the cocci (especially the nonhemolytic Streptococcus) and the Bacteroides group (especially Bacteroides melaninogenicus). Except for E. coli, none of these organisms is anything but a weak producer of gas. E. coli rapidly ferments sugar to produce lactic acid, carbon dioxide, and hydrogen. The fermentation process is aided by anaerobic conditions and abetted by the organisms mentioned above, which act synergistically with E. coli.

The three cases presented illustrate the above-mentioned points. In Case 1, a clysis given in the thigh to a diabetic in coma was the source of entry of E. coli. A gas infection resulted. In Case 2, the infection followed the removal of a gangrenous appendix and was also due to E. coli. In Case 3, a perianal abscess in an unsuspected diabetic resulted in a gas infection and, finally, death in coma because of an uncontrolled diabetic state. Gram-positive cocci plus Gram-negative and Gram-positive bacilli were the offending organisms.

It is thus seen that the precise bacterial cause of the infection is of less importance than the determination of the absence of clostridia. There are reports of cases in which conditions were diagnosed and treated as gas gangrene, although repeated adequate cultures failed to yield clostridia. It is well to remember that such cases may not be of gas gangrene at all.

Since the combination of infection, high tissue sugar, and impaired circulation is so common, it is strange that the condition described in this paper is not more frequent. The explanation may lie in the fact that the E. coli is only rarely the cause of suppurative inflammation in the extremities.

Multiple incisions, both in gas gangrene and in nonclostridial gas infections, to free the tissues of gas and pus and the administration of antibiotics are the treatment of choice. The prognosis in nonclostridial gas infection is excellent, provided the condition is recognized and adequate treatment instituted.

## CONCLUSION

It is the purpose of this communication to bring to the attention of the medical profession the fact that the presence of gas in infected tissue, especially in a diabetic person, does not necessarily mean anaerobic clostridial gas gangrene with its concomitant poor prognosis but is very likely due to other gas-producing organisms, such as Escherichia coli, and has a good prognosis with conservative surgical and antibiotic therapy.

Dr. M. F. Levinson and Dr. Sidney Cohn gave permission for use of reports of their cases.

# CLINICAL SIGNIFICANCE OF PLEUROPERICARDIAL CYST

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PLEUROPERICARDIAL and pleurocoelomic are synonymous terms applied to a thin-walled cystic structure, typically lying on or near the diaphragm in the right cardiophrenic space. Attempts have been made to classify pleuropericardial cysts with other simple cysts of the pleura; however, their mode of origin and location appear to be specific, and they are, therefore, assigned to a separate category.

Pleuropericardial cysts must have a much higher incidence than is evidenced by the infrequency with which they have been reported. Johnson [1] stated that he supposed the reason few reports of these cysts appear in the literature is that they are of so little significance in themselves that surgeons seldom feel them worthy of discussion. So far as the patient is concerned this may be true, but from the viewpoint of the chest physician the establishment of the diagnosis is not always simple, and, until definitely classified, a pleuropericardial cyst can be confused with a number of really important chest tumors. In 1936 Andrus and Heuer [2] stated that only 15 cases of cysts of the mediastinum, other than dermoids and teratomas, had been reported in the literature. In 1946 Blades [3] reported on 114 thoracotomies done in Army Thoracic Surgery Centers for tumors of various kinds, and of these tumors 10 were pleuropericardial cysts. It is presumed that these cases include the "eight or ten cases" cited by Maier [4] in a personal communication as having been noted at Kennedy General Hospital during the war. Burnett,[5] Wilson,[6] and Majors [7] have each stated that they had seen two pleuropericardial cysts in the past, while Johnson [1]

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Read before the Scientific Meeting of the Columbia Medical Society at Columbia, S. C., Feb. 12, 1951.

Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Johnson, J.: Personal communication to the authors.
2. Andrus, W. D., and Heuer, G. J.: Surgical Treatment of Tumors of the Mediastinum, Surg., Gynec. & Obst. **63**:469-482 (Oct.) 1936.
3. Blades, B.: Mediastinal Tumors, Ann. Surg. **123**:749-765 (May) 1946.
4. Maier, H. C.: Personal communication to the authors.
5. Burnett, W. E.: Personal communication to the authors.
6. Wilson, J. L.: Personal communication to the authors.
7. Majors, R. C.: Personal communication to the authors.

said he believed he had operated in four cases of such chest tumors. Edwards [8] during 1926 and d'Abreu [9] in 1937 reported in British surgical journals on pleurocardial cysts, while Nelson [10] in 1932, Freedman and Simon [11] in 1936, and Addey [12] in 1940 reported in journals of this country on similar cysts. Recently, Drash and Hyer [13] collected 16 cases from the literature and added five cases in which they had performed surgical procedures. It is evident from this survey that pleuropericardial cysts do not occur too commonly; yet, in this day of extensive chest surveys in connection with tuberculosis control, these almost asymptomatic tumors of the chest may be noted with increasing frequency and in each instance require special differential diagnosis.

## ETIOLOGICAL ASPECTS

The etiology of the pleuropericardial cyst is poorly understood. It has been stated by some writers that these cysts are the result of proliferation of misplaced cells of either the digestive or the respiratory system during embryonic migration. Boyd [14] expressed the opinion that the cysts arise from diverticula of the pleura which become sealed off and increase in size. Pund's [15] statement that they represent the remains of the congenital pleuropericardial sinus, and are, therefore, found on the right side at the cardiophrenic angle, is most plausible. Johnson [1] apparently holds a similar opinion. In his experience the pleuropericardial cysts are of two types: The one, simulating a pericardial herniation, communicates with the pericardial cavity, and the contained fluid can be expressed into the pericardial space; the other is completely separate from the pericardium but lies adjacent to it.

A careful study of the subject by Lambert [16] indicates that the pericardium arises from a series of disconnected lacunas which appear in early embryonic life. These lacunas are first seen in the mesenchyma lateral and ventral to the primitive streak. The spaces enlarge and fuse to form the horseshoe-shaped pericardial coelom. At first this is an independent cavity, but eventually it communicates with the pleuroperitoneal coelom. Subsequently this communication is closed by the ligamentum transversum and ducts of Cuvier. Failure in closure of the communication between the pericardial and pleuroperitoneal coeloms causes congenital absence of the pericardium. Failure of the ligamentum transversum to divide the pleuroperitoneal coelom into pleural and peritoneal cavities causes congenital diaphragmatic hernia. Inequality in the rate of development of the mesenchymal-pericardial lacunas causes

8. Edwards, A. T.: Intrathoracic New Growths: Account of 7 Operable Cases, Brit. J. Surg. **14**:607-608 (April) 1927; cited by Drash, E. C., and Hyer, H. J.: Mesothelial Mediastinal Cysts, J. Thoracic Surg. **19**:755-768 (May) 1950.

9. d'Abreu, A. L.: Primary Pleural Cysts, Brit. J. Surg. **25**:317-322 (Oct.) 1937.

10. Nelson, R. L.: Congenital Cystic Disease of Lung: Report of Case, J. Pediat. **1**:233-238 (Aug.) 1932.

11. Freedman, E., and Simon, M. A.: Simple Cyst of the Pleura: Report of a Case, Am. J. Roentgenol. **35**:53-56 (Jan.) 1936.

12. Addey, W.: A Case of Pleural Cyst, Brit. J. Radiol. **13**:180-182 (May) 1940.

13. Drash, E. C., and Hyer, H. J.: Mesothelial Mediastinal Cysts, J. Thoracic Surg. **19**:755-768 (May) 1950.

14. Boyd, W.: Surgical Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1947.

15. Pund, E. R.: Personal communication to the authors.

16. Lambert, cited by Gillespie, S. R., and Martinson, L. F.: Pericardial Celomic Cysts, Northwest Med. **49**:107-110 (Feb.) 1950.

congenital diverticulum of the pericardium. It is probable that pleurocoelomic or pleuropericardial cyst is caused by failure of one of the primitive lacunas to merge with the others.

## HISTOLOGICAL AND PATHOLOGICAL CHARACTERISTICS

The characteristic histological picture of the pleuropericardial cyst is that of a thin-walled fibrous-tissue sac covered by the parietal pleura and lined with a very thin endothelium. The cystic fluid is like that normally encountered in the pericardium, being relatively free of cells and of low specific gravity.

Although, pathologically speaking, a pleuropericardial cyst may be of little consequence, other tumors from which it must be differentiated are significant. Of these tumors, teratomas and dermoids are the most frequent, while lipomas, chondromas, lymphangiomas, sarcomas, pleural endotheliomas, calcified hematomas, metastatic carcinomas, and secondary hydatid cysts must be considered. It is also possible for a diaphragmatic hernia or a pleural effusion to be simulated by one of these cysts. Diagnostic methods include induced pneumothorax and roentgenography, angiocardiography, bronchography, and needle puncture. Pneumoperitoneum can be resorted to when one suspects a diaphragmatic hernia or subphrenic abscess. Occasionally, the difficulty in establishing a diagnosis will necessitate an exploratory thoracotomy, at which time, if a pleuropericardial cyst is discovered, extirpation of the cyst is relatively easily performed. Three instances of pleuropericardial cyst recently encountered at the Veterans Administration Hospital, Columbia, S. C., in which operation was performed are described as typical examples of the condition.

## REPORT OF CASES

Case 1 (66979).—A 33-yr.-old white man was admitted to the Columbia Veterans Administration Hospital, Sept. 20, 1949, because he noted fatigability and shortness of breath for several months. He had continued his regular work until three weeks before hospitalization, when a tumor near the base of the right lung was discovered on roentgenographic examination during a tuberculosis survey. Except for dyspnea and fatigability, there were no other symptoms referable to the chest. A routine complete physical examination revealed no significant abnormalities. Results of laboratory examinations were all within normal limits, including absence of tuberculosis organisms on tests of sputum and a negative reaction to the Vollmer patch test. The roentgenographic examination revealed a sharply circumscribed density (Fig. 1) in the right cardiophrenic angle, measuring approximately 8 cm. in diameter. It was continuous with the cardiac silhouette anteriorly beneath the chest wall. Diagnostic pneumothorax showed the mass to be outside the lung parenchyma, but not separate from the anterior chest wall and mediastinum (Fig. 2). An attempt to aspirate this mass was unsuccessful. A thoracotomy was performed, and a pleuropericardial cyst was observed. The pleura was incised near its base, and the cyst was stripped from its attachments to the chest wall and the epicardial fat in the mediastinum. The patient recovered from the procedure without delay, and subsequent roentgenographic survey of the chest showed a normal condition except for evidence of a rib resection. In this instance the cyst wall was observed to be a thin fibrous-tissue structure lined by a thin endothelium. The cyst contained a clear fluid having a specific gravity of 1.004 and a protein content of 1.2 gm. per 100 cc.

Case 2 (64015).—A 57-yr.-old Negro man was admitted to the Columbia Veterans Administration Hospital in July, 1948, with the complaint of pain in the chest. He was observed to have generalized rheumatoid arthritis and a blood pressure of 170/110. A roentgenogram of the chest showed a globular mass in the right lower lung field. A bronchoscopic examination was

Fig. 1 (Case 1).—Lateral (left) and posteroanterior (right) projections of chest.

Fig. 2 (Case 1).—Diagnostic pneumothorax. Lower and middle lobes of right lung have collapsed away from mass, demonstrating its extrapulmonary location.

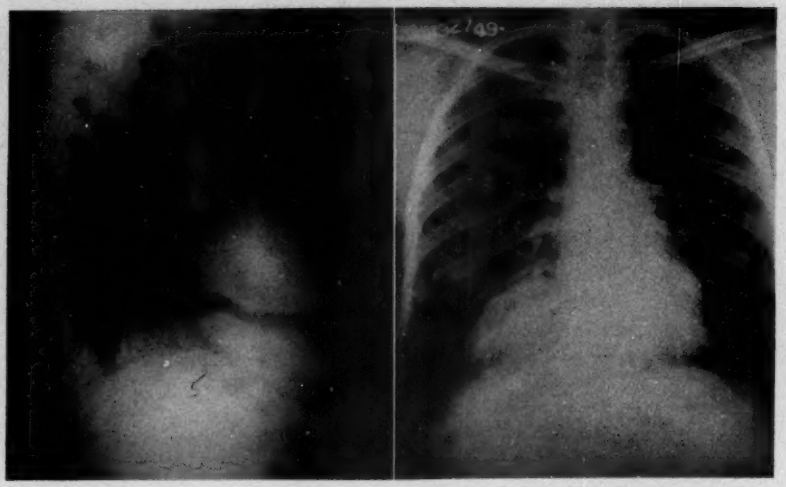

Fig. 3 (Case 2).—Lateral (left) and posteroanterior (right) projections of chest. In lateral projection pneumothorax has been performed, demonstrating supradiaphragmatic position of density.

Fig. 4 (Case 3).—Posteroanterior projection of chest. Density is indistinguishable from infrapulmonary pleural effusion. Position of diaphragm cannot be determined.

proposed and refused by the patient, who promptly left the hospital. In April, 1949, the patient returned to the hospital, stating that in the interval he had continued to have pain in the chest and a cough, sometimes productive of phlegm, but not of blood. The history was otherwise not significant, except for a weight loss of 11 lb. (5 kg.). Physical examination revealed nothing of note except moderate hypertension and diffuse arthritis, with painful and limited motion of all joints. Laboratory examinations gave normal results, except for a 3+ reaction to a Kahn blood test and 100% complement fixation. The chest roentgenographic examination revealed a homogeneous, smooth, lobulated mass at the base of the right lung field, obscuring the right cardiophrenic angle (Fig. 3). The remainder of the lungs was normal. The heart was moderately enlarged and the aorta tortuous. A right pneumothorax performed at this time showed a thin streak of air along the surface of the tumor mass, indicating its extrapulmonary location. The mass could not be separated from the mediastinal structures.

An exploratory thoracotomy was performed through the eighth rib on the right side. A cyst 8 cm. in diameter was discovered. The cyst was excised by a combination of sharp and blunt

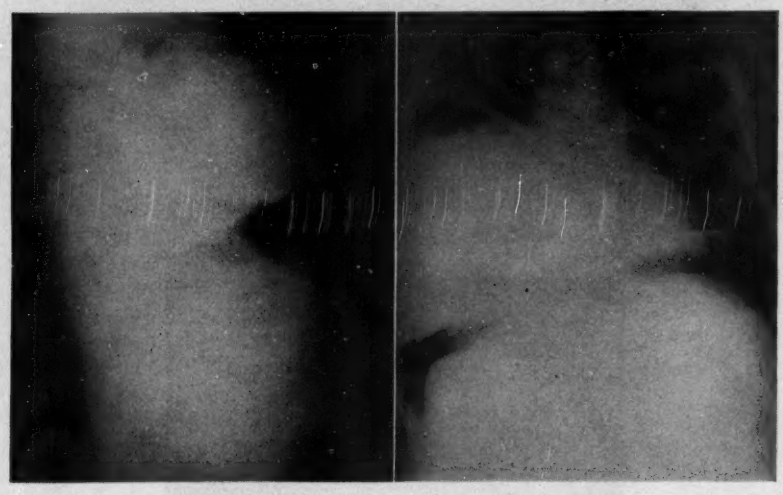

Fig. 5 (Case 3).—Lateral (left) and posteroanterior (right) projections of chest after pneumoperitoneum. Position of density is seen to be entirely supradiaphragmatic.

dissection. The postoperative recovery was uneventful. After operation roentgenographic examination revealed slight pleural thickening at the base of the right lung; otherwise the lung fields were normal. Pathological examination revealed a multilocular cyst, having a thin, glistening wall of hyaline fibrous connective tissue lined with a thin layer of endothelial-like cells. The cystic fluid was clear, yellow tinged, and slightly viscid. The fluid was spilled; hence it was not analyzed.

Case 3 (74716).—A 40-yr.-old white man was admitted to the Columbia Veterans Administration Hospital, Nov. 11, 1950, complaining of a pain which began in the right flank, migrated to the right posterior portion of the chest, and radiated into the right epigastrium. This complaint had its onset six weeks prior to hospital admission. There was no other pertinent history. The physical examination was unrevealing except for the chest findings. There was absence of tactile fremitus at the base of the right lung, associated with dulness on percussion and absence of breath sounds, but no rales could be heard. On roentgenographic examination

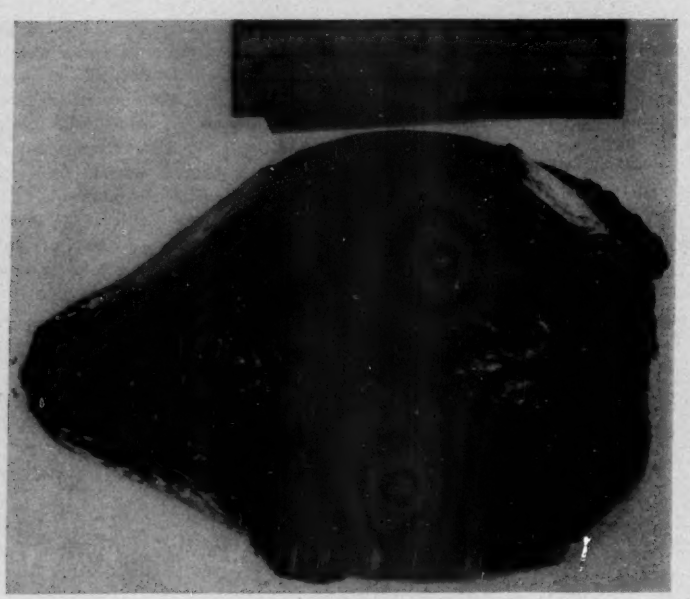

Fig. 6 (Case 3).—Gross appearance of pleuropericardial cyst on excision.

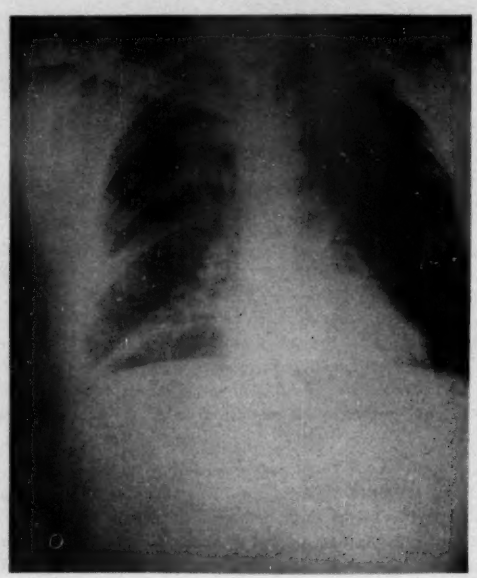

Fig. 7 (Case 3).—Postoperative roentgenogram.

a large spherical mass, measuring 17.5 cm. in diameter, occupied the lower half of the right pleural space (Fig. 4). There was evidence of atelectasis of the lower part of the right lung and thickening of the interlobar fissure. Laboratory examinations revealed normal blood counts and normal results on liver-tests and kidney-function tests. At this point a tentative diagnosis of an encapsulated pleural effusion, or possibly a subphrenic abscess, was made. Pneumoperitoneum was performed, and on roentgenographic examination a free subdiaphragmatic space was seen, with slight depression of the diaphragm (Fig. 5). An aspiration of the cyst through the sixth interspace on the right in the midaxillary line revealed a collection of clear fluid with specific gravity of 1.007. This fluid contained 9 white blood cells per high-power field, of which 7 were lymphocytes and 2 polymorphonuclear forms. The total protein content was 2.12 gm. per 100 cc. No organisms were noted. At this point, a diagnosis of pleuropericardial cyst was made and preparation for thoracotomy initiated. At operation a pear-shaped cyst measuring 16.2 by 11.3 cm. was removed (Fig. 6). This cyst entirely filled the lower right pleural cavity and was fixed by adhesions posteriorly. The anterior attachment was to the pericardium in the right cardiophrenic angle. The microscopic examination revealed that the cyst wall was composed of fibrous connective tissue lined by flattened cells containing widely separated small dark-staining nuclei. The postoperative course was essentially unremarkable. The lung reinflated, and the wound healed cleanly. Postoperative follow-up study showed uneventful recovery and a grossly normal chest roentgenogram (Fig. 7). An incidental observation at operation was that the diaphragm was depressed and did not move with respiration. Postoperative study showed the diaphragm at approximately its normal level but still not moving with respiration.

## COMMENT

Although it is readily admitted that a pleuropericardial cyst in itself is of little pathological importance, the cyst is clinically significant in that it must be clearly differentiated from a large number of other pleural space-occupying masses appearing in the same region which are of particular pathological consideration. The examination of a person suspected to have pleuropericardial cyst may require the use of contrast roentgenograms employing pneumothorax and pneumoperitoneum and special iodine preparations for angiocardiography. When the aspiration of pleuropericardial cyst reveals the typical fluid, it is diagnostic, but here we feel one must proceed with caution because, should the pleural mass prove to be a malignant tumor, tumor cells may be implanted into the pleural space or along the track of the puncture wound in the chest wall. When a recent pulmonary infection is associated with the presence of a large pleuropericardial cyst, as in the third case presented in this paper, the signs are not unlike those of an encapsulated pleural effusion, especially when atelectasis and pleurisy are demonstrable. Cushing[17] in 1937 published a report on a series of cases of pericardial diverticulum collected from the literature. This condition might also be easily confused with pleuropericardial cyst, particularly in the presence of pericardial effusion of pyogenic or tuberculous origin. Surgical treatment is not necessarily indicated for the unequivocally diagnosed small pleuropericardial cyst, but when the mass has attained sufficient size to cause respiratory distress of any degree the relatively innocuous procedure required for its extirpation should be advised. Recovery is usually uneventful after operation, and the appearance of a relatively normal postoperative chest roentgenogram is gratifying.

17. Cushing, E. H.: Diverticulum of the Pericardium, Arch. Int. Med. **59**:56-64 (Jan.) 1937.

SUMMARY

Pleuropericardial or pleurocoelomic cyst is a thin-walled structure, typically occupying the right cardiophrenic angle. It is covered by pleura and lined by endothelial-like cells, whereas the wall itself is made up of poorly defined fibrous tissue. The cystic fluid is not unlike that encountered in the pericardium, having a low specific gravity, low protein content, and few, if any, leucocytes. The mode of origin is not specifically understood, but careful comparison of opinions indicates the acceptance of a theory that the cyst results from a developmental irregularity of the lacunas in the primitive mesenchyma. Relatively few cases of such cysts have been reported in the literature, although there is evidence that they must occur fairly frequently. During the course of tuberculosis surveys, they will no doubt often be seen, and it will require careful differential diagnosis in most instances to separate this relatively innocuous cyst from a large number of similar-appearing tumors of the chest. Roentgenologic examination, employing pneumothorax, pneumoperitoneum, bronchography, and angiocardiography, is the principal means of diagnosis; however, in selected cases, aspiration may give conclusive evidence. Surgical treatment is indicated when the cyst becomes so large as to cause dyspnea and pain, and when differential diagnosis fails to show conclusively the character of the tumor.

Three surgically proved cases of pleuropericardial cyst are presented, with a description of the clinical methods used in arriving at the diagnosis.

# METABOLIC PROBLEMS ARISING IN THE MANAGEMENT OF CONGESTIVE HEART FAILURE

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ST. LOUIS

THREE patients who received conventional therapy for heart failure at the St. Louis City Hospital regained compensation but died within a few days without apparent cause. Anorexia, lethargy, and, eventually, stupor preceded the death of each. Analysis of their records and clinical course suggested that an electrolyte imbalance had been precipitated during the treatment of the cardiac failure. Later, when similar patients were treated in a comparable manner, acidosis was discovered and dramatically corrected by the judicious use of sodium lactate. This experience led us to study the blood chemical pattern in patients with congestive heart failure before and during treatment with digitalis, ammonium chloride, mercurial diuretics, and a low-salt diet.

## METHOD OF STUDY

Twenty-four patients in severe congestive heart failure were studied during the winter months when environmental temperatures could be controlled. Most of these patients had hypertensive or arteriosclerotic heart disease, or both. An attempt was made to exclude those patients with primary renal disease. Before therapy the following laboratory determinations were made: blood urea nitrogen, serum chloride, serum carbon dioxide-combining power, and, in several cases, serum sodium, serum potassium,[1] and venous pH.[2] These were redetermined at three- to four-day intervals thereafter. Except for three patients who had ammonium chloride acidosis when admitted to the hospital, therapy consisted of digitalis, 6 gm. of ammonium chloride (enteric-coated®) daily, and 2 ml. of mersalyl (salyrgan®) daily for three or four days. Further use of the last-named drug was determined by the amount of remaining or recurring edema. The daily diet contained 3 gm. of sodium chloride. Diuresis was estimated from daily weight determinations instead of by measurement of urinary output. No patient received lithium salt substitutes.

## RESULTS

Of the 24 patients admitted with congestive heart failure, 13 had received no recent cardiac therapy. In the latter group, therefore, it was possible to study the electrolyte pattern and acid-base balance in untreated heart failure (Table 1). Many

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1. The Weichselbaum-Varney flame spectrophotometer was used through the courtesy of the Metabolic Laboratory of Barnes Hospital.

2. Hastings, A. B., and Sendroy, J., Jr.: Studies of Acidosis: XX. The Colorimetric Determination of Blood pH at Body Temperature Without Buffer Standards, J. Biol. Chem. **61**:695-710 (Oct.) 1924.

of the patients showed little deviation from the normal. Three members of this group had slight nitrogen retention; three had slightly elevated serum chloride levels. Likewise, the carbon dioxide-combining power was normal in all except P. G., a man who had emphysema. In the five patients on whom serum pH studies were made, the values varied from 7.38 to 7.5. Serum sodium levels of 148 mEq. or more were seen in three subjects with emphysema and in one with bilateral pleural effusion. A low serum sodium value (132 mEq.) was observed in only one case. Six patients showed a potassium level above 5 mEq.

Twenty-one patients of the 24 studied received intensive treatment for congestive heart failure. About one-third of them tolerated a severe diuresis and a prolonged use of diuretics without displaying uremia or acidosis, even though the weight loss in several instances was as great as 14 to 20 kg. Another third of the group showed slight rises in the blood urea nitrogen, to values of 35 to 39 mg. per 100 ml., during the process of dehydration. A spontaneous fall to the normal occurred while they were still receiving treatment (Case 1). A third group, as represented by Case 2, showed moderately severe acidosis and nitrogen retention. It is interesting that one

TABLE 1.—*Laboratory Data on Admission of Thirteen Patients with Congestive Heart Failure*

| Name | Age | Sex | Blood Urea Nitrogen, Mg./100 Ml. | mEq./L. $CO_2$ Combining Power | Chloride | Sodium | Potassium | pH | Comment |
|---|---|---|---|---|---|---|---|---|---|
| P. G. | 68 | M | 15 | 32.4 | 103 | 154 | 5.2 | ... | Emphysema |
| A. N. | 76 | M | 19 | 28.8 | 106 | 152 | ... | ... | Emphysema |
| A. P. | 75 | F | 25 | 24.3 | 107 | 150 | ... | ... | Bilateral pleural effusions |
| N. S. | 61 | F | 12 | 27.0 | 99 | 148 | 5.7 | ... | Moderate emphysema |
| R. S. | 65 | M | 22 | 29.7 | 103 | 140 | 5.5 | 7.5 | No therapy |
| A. F. | 75 | M | 12 | 29.7 | 103 | 145 | 4.7 | ... | No therapy |
| M. S. | 63 | M | 20 | 30.6 | 105 | ... | ... | ... | No therapy |
| D. W. | 62 | F | 12 | 19.8 | 101 | 146 | 4.7 | ... | No therapy |
| N. D. | 65 | F | 13 | 23.8 | 108 | 143 | 6.5 | 7.48 | No therapy |
| D. A. | .. | F | 18 | 28.4 | 102 | 139 | 4.4 | ... | No therapy |
| F. H. | 76 | M | 36 | 23.4 | 97 | 152 | 5.3 | 7.48 | No therapy |
| H. G. | 74 | M | 13 | 26.1 | 98 | 136 | 5.1 | 7.48 | No therapy |
| J. W. | 50 | M | 13 | 24.8 | 108 | ... | ... | 7.38 | No therapy |

of these patients (Case 3) had laboratory evidence of acidosis, though clinically he was alert and felt well.

CASE 1.—R. S., a man 65 yr. of age, entered the hospital because of increasing dyspnea and orthopnea during the previous three weeks. The blood pressure was 135/90, the pulse rate 108, and the respiratory rate 22. Cardiac enlargement, pulmonary congestion with pleural effusion, hepatomegaly, and marked dependent edema were present. Serial electrocardiograms demonstrated an anteroseptal infarction which was considered inactive. The urinalysis made on admission revealed a specific gravity of 1.004, pH 5.5, and a negative sediment. A sulfobromophthalein (bromsulphalein®) sodium test (with administration of 5 mg. per kilogram of body weight) showed 9% retention in 45 min.

Ammonium chloride was given daily until the 25th day. Ten injections of mersalyl were given intravenously during the first two weeks. Within the first week the patient lost 7 kg. and thereafter kept a fairly constant weight. The blood urea nitrogen slowly rose from 22 mg. to 39 mg. per 100 ml. and then dropped to 12 mg. spontaneously (Table 2). No significant change occurred in the blood pH or electrolytes. The patient was discharged on the 33d hospital day in good condition.

CASE 2.—J. W., a man 50 yr. of age with hypertensive and arteriosclerotic heart disease, reentered the hospital because of progressive symptoms of cardiac decompensation after omission of digitalis administration 10 weeks previously. The blood pressure was 155/100, and the pulse rate was 112. Distention of the neck veins, pulmonary congestion, liver enlargement, and edema

were present. Urinalysis showed a specific gravity of 1.015, pH 5.5, and no abnormality of sediment. All other laboratory data were likewise normal (Table 3).

He was digitalized, given a salt-free diet, and for the first 15 days received mersalyl and ammonium chloride daily. His signs of heart failure disappeared, and by the 11th day he had lost 21 kg. of body weight. On this day the patient manifested weakness, somnolence, anorexia, apathy, and irritability. On his 15th hospital day, the blood urea nitrogen had risen to 115 mg. per 100 ml. and blood studies revealed an acidosis (blood pH, 7.26; carbon dioxide-combining power, 14.9 mEq.). The administration of diuretics was promptly stopped, and 1 l. of M/6 sodium lactate solution was given. The acidosis was corrected within 24 hr. but the azotemia did not disappear until the fourth day of treatment. The patient was discharged with good cardiac compensation three days later. One year later, the patient died with a coronary occlusion. Autopsy revealed no disease of the liver or kidneys.

CASE 3.—H. G., a man 73 yr. of age with arteriosclerotic heart disease, was admitted to the hospital because of increasing dyspnea and pains in the chest for 10 days and dependent edema

TABLE 2.—*Summary of Clinical Data on Patient R. S. (Case 1)*

| Day | Weight, Kg. | Blood Urea Nitrogen, Mg./100 Ml. | mEq./L. $CO_2$ Combining Power | Chloride | Sodium | Potassium | pH |
|---|---|---|---|---|---|---|---|
| 1 | 75 | 22 | 29.7 | 103 | 140 | 5.5 | 7.5 |
| 5 | 62.3 | 14 | 27.0 | 104 | ... | ... | ... |
| 8 | 57.3 | 37 | 25.2 | 106 | ... | ... | ... |
| 10 | 59.1 | 33 | 22.5 | 117 | ... | ... | ... |
| 12 | 58.2 | 32 | 25.7 | 105 | ... | ... | ... |
| 15 | 50.1 | 30 | 22.5 | 109 | 145 | 5.1 | 7.42 |
| 19 | .... | 22 | 21.2 | 114 | ... | ... | ... |
| 22 | 56.4 | 35 | 22.5 | 106 | 142 | 5.8 | ... |
| 24 | 55.5 | 39 | 24.8 | 109 | ... | ... | 7.42 |
| 26 | 56.4 | 24 | 22.5 | 109 | ... | ... | ... |
| 29 | 57.3 | 17 | 28.8 | 102 | 150 | 5.1 | 7.50 |
| 33 | 57.7 | 12 | 31.1 | 99 | ... | ... | 7.48 |

TABLE 3.—*Summary of Clinical Data on Patient J. W. (Case 2)*

| Day | Weight, Kg. | Blood Urea Nitrogen, Mg./100 Ml. | mEq./L. $CO_2$ Combining Power | Chloride | Sodium | Potassium | pH |
|---|---|---|---|---|---|---|---|
| 1 | 78 | 13 | 24.8 | 108 | ... | ... | 7.38 |
| 5 | 64 | 14 | 29.3 | 101 | ... | ... | .... |
| 10 | 61 | 40 | 20.7 | 108 | 134 | 5.9 | 7.40 |
| 12 | 59 | 72 | 22.8 | 101 | 136 | 6.2 | 7.31 |
| 13 | 59 | 76 | 16.7 | ... | ... | ... | ... |
| 15 | 57 | 116 | 14.9 | 107 | ... | ... | 7.26 |
| 16 | 58 | 116 | 19.4 | 102 | 143 | 5.4 | 7.45 |
| 22 | 61 | 27 | 23.8 | 106 | 143 | 6.3 | 7.48 |

for five days. The blood pressure was 135/60, the pulse rate 94, and the respiratory rate 22. The patient was in moderate respiratory distress and showed slight cyanosis. In addition to the usual signs of severe heart failure, bilateral pleural effusions were present. Urinalysis on admission showed a specific gravity of 1.007, an acid reaction, and a negative sediment. The blood urea nitrogen and electrolytes were normal (Table 4).

He received ammonium chloride daily and mersalyl almost daily for the first 18 days. He lost 15 kg. of body weight in 16 days. He showed an increasing nitrogen retention and a decreasing carbon dioxide-combining power. On the 20th hospital day he was in moderate acidosis, though he appeared well. Administration of diuretics was discontinued, and oral administration of fluids was forced. The acidosis was corrected, and the patient was discharged, well compensated, on his 27th hospital day.

Three of the 24 patients were hospitalized because of semistupor and mental confusion due to acidosis and uremia from the use of ammonium chloride. On admission these persons (represented by Cases 4 and 5) showed no signs of heart

failure, although they had been previously in a state of cardiac decompensation. They had been given a low-salt diet at home and had received ammonium chloride (3 to 8 gm.) daily and no mercurial diuretics. These patients had a high blood chloride level and a low serum carbon dioxide-combining power, which in one patient was 7.7 mEq. The patient with this value likewise had the exceedingly low serum pH of 7.1. The sodium value was determined in two of the subjects and was found to be slightly reduced (131 and 136 mEq.), while the serum potassium was at the upper limits of normal. During the rehydration of one patient (Case 5), the potassium dropped to subnormal levels without producing any obvious symptoms.

CASE 4.—M. E., a woman 68 yr. of age, was seen for the first time in the hospital clinic, where her condition was diagnosed as mild cardiac decompensation. She was given a low-salt

TABLE 4.—*Summary of Clinical Data on Patient H. G. (Case 3)*

| Day | Weight, Kg. | Blood Urea Nitrogen, Mg./100 Ml. | mEq./L. | | | | pH |
|---|---|---|---|---|---|---|---|
| | | | $CO_2$ Combining Power | Chloride | Sodium | Potassium | |
| 1 | 67 | 13 | 26.1 | 98 | 136 | 5.1 | 7.48 |
| 6 | 64.5 | 15 | 26.6 | 91 | ... | ... | .... |
| 9 | 57.3 | 18 | 27.0 | 104 | 140 | 5.6 | 7.45 |
| 12 | 56 | 28 | 25.2 | 97 | ... | ... | .... |
| 16 | 52.7 | 39 | 20.2 | 101 | 136 | 5.9 | 7.41 |
| 18 | 52 | 44 | 19.9 | 100 | ... | ... | .... |
| 20 | 53.6 | 42 | 15.8 | 99 | 122 | 6.6 | 7.30 |
| 21 | .. | 42 | 22.0 | 98 | ... | ... | .... |
| 23 | 52.7 | 20 | 27.9 | 95 | 132 | 6.4 | 7.42 |
| 27 | 52.3 | 20 | 30.1 | 97 | ... | ... | 7.48 |

TABLE 5.—*Summary of Clinical Data on Patient M. E. (Case 4)*

| Day | Blood Urea Nitrogen, Mg./100 Ml. | mEq./L. | | | | Therapy | |
|---|---|---|---|---|---|---|---|
| | | $CO_2$ Combining Power | Chloride | Sodium | Potassium | Amount, Ml. | Solution |
| 1 | 52 | 15.8 | 115 | 131 | 4.8 | 1,000 | M/6 sodium lactate |
| 2 | 60 | 18.5 | 104 | ... | ... | 2,000 | 5% dextrose |
| | | | | | | 1,000 | 10% dextrose |
| | | | | | | 1,000 | M/6 sodium lactate |
| 3 | .. | 26.6 | 96 | ... | ... | 1,000 | 10% dextrose |
| | | | | | | 1,000 | 5% dextrose |
| 4 | 32 | .... | ... | ... | ... | 1,000 | 10% dextrose |
| 5 | 24 | 27.9 | 104 | 143 | 4.8 | ..... | ..... |
| 13 | 20 | 27.0 | 108 | 132 | 5.7 | ..... | ..... |

diet and ammonium chloride (8 gm.) daily. This regimen was continued for 12 days, when she entered the hospital because of weakness and delusions. On admission the respirations were "deep and sighing," with the rate of 24. The blood pressure initially was 164/84 but later stabilized at 200/110. The heart was well compensated, but its size was at the upper limits of normal. The neurologist made a diagnosis of organic psychosis due either to uremia or to cerebral arteriosclerosis. Urinalysis revealed a specific gravity of 1.012, pH 5, a trace of sugar and albumin, and a negative sediment. The initial blood studies showed the following values expressed as milliequivalents per liter: chloride, 115; carbon dioxide-combining power, 15.8; sodium, 131; potassium, 4.8. The blood urea nitrogen was 52 mg. per 100 ml. (Table 5).

After therapy with 2.1 of M/6 sodium lactate solution, the acidosis was corrected. Concomitantly, the psychotic manifestations disappeared, and she was discharged on her 13th hospital day in good condition. On subsequent visits to the clinic, the patient remained mentally clear.

CASE 5.—J. H., a man 65 yr. of age, entered the hospital with weakness, nausea, anorexia, confusion, and hallucinations. Because of cardiac decompensation due to hypertensive cardiovascular disease, he had been receiving digitalis, 0.1 gm. daily, and ammonium chloride in vary-

ing amounts (up to 6 gm. daily) until 12 days prior to admission, when the dose of the latter was changed to 3 gm. daily. Weakness and anorexia had begun three weeks before hospitalization, and the psychotic behavior was a more recent development. No complaints were referable to the cardiovascular system. The blood pressure was 155/85, the pulse rate 88, and the respiratory rate 20. Heart and liver enlargement was present, but the chest was clear. The reflexes were active and equal. The urinalysis on admission revealed a specific gravity of 1.010, pH 5.3, 2 + reaction for albumin, and a negative sediment.

Table 6 shows the blood chemical pattern on admission and during treatment. The calcium level was 8.3 mg., and the phosphorus value was 7.4 mg. per 100 ml. Several days after admission, the cephalin-cholesterol flocculation reaction was 3 + in 48 hr., and the sulfobromophthalein test showed 25% retention in 45 min. Parenteral administration of large amounts of M/6 sodium lactate solution and other fluids was required before the acidosis, uremia, and symptoms were relieved. The patient was discharged with good cardiac compensation on the 25th hospital day.

A review of the hospital record of this patient revealed that four months previously a diagnosis of psychosis due to phenobarbital intoxication had been made. Hallucinations, anorexia, and nausea had occurred 10 days after phenobarbital (0.03 gm. four times daily) had been prescribed. The patient had been receiving ammonium chloride at the same time. He

TABLE 6.—*Summary of Clinical Data on Patient J. H. (Case 5)*

| Day | Body Weight, Kg. | Blood Urea Nitrogen, Mg./100 Ml. | mEq./L. | | | | pH | Therapy | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ Combining Power | Chloride | Sodium | Potassium | | Amount, Ml. | Solution |
| 1 | .. | 128 | 7.7 | 114 | 136 | 4.7 | 7.1 | 2,500<br>500 | M/6 sodium lactate<br>5% dextrose |
| 2 | .. | 144 | 17.1 | 107 | 139 | 3.5 | 7.36 | 750<br>1,250 | M/6 sodium lactate<br>5% dextrose |
| 3 | .. | 122 | 17.1 | 106 | 139 | 3.4 | 7.38 | 750<br>2,250 | M/6 sodium lactate<br>5% dextrose |
| 4 | .. | 129 | 21.6 | 103 | ... | ... | .... | 1,000<br>2,000 | 10% dextrose<br>5% dextrose |
| 5 | 59 | 104 | 23.8 | 96 | 138 | 2.8 | 7.45 | 2,000<br>500<br>500 | 5% dextrose<br>Isotonic sodium chloride<br>Blood |
| 6 | .. | 80 | 22.8 | 98 | ... | ... | .... | 1,000<br>500<br>500 | 5% dextrose<br>Isotonic sodium chloride<br>Blood |
| 7 | .. | ... | .... | ... | ... | ... | .... | 3,000 | 5% dextrose |
| 8 | .. | 52 | 25.2 | 91 | 133 | 2.8 | 7.58 | 2,000<br>1,000 | 5% dextrose<br>Isotonic sodium chloride |
| 16 | 62 | 30 | 26.1 | 101 | 145 | 4.4 | 7.48 | ..... | ..... |

attributed his symptoms to the ammonium chloride and discontinued using it but continued taking the phenobarbital. According to the family, the mental symptoms cleared partially within the next three days. Nevertheless, the patient was hospitalized. On admission he was slightly confused, though not hallucinating. The blood urea nitrogen was 60 mg. per 100 ml., the chlorides 509 mg. per 100 ml. (whole blood), and the carbon dioxide-combining power 19 mEq. per liter. After parenteral administration of isotonic sodium chloride solution, the patient became "alert and talkative." The blood urea nitrogen fell to 23 mg. per 100 ml. on discharge on the 13th hospital day. Presumably, the correct diagnosis for this illness was subsiding acidosis due to ammonium chloride.

The use of mercurial diuretics without supplementary ammonium chloride was studied in only one patient (Case 6). After he had a fair diuresis from several injections of mersalyl, his condition gradually failed to respond to use of the drug, though edema remained moderately severe. He lost his appetite and became mentally confused; he had no complaints of abdominal or leg cramps. A sodium chloride deficit was suspected, and his serum chloride level was found to be 69 mEq. per liter at a time that his carbon dioxide-combining power was 31.5 mEq. Unfortunately, serum sodium levels could not be obtained in this case. Within 24 hr. after the infusion

of hypertonic saline solution, his serum chloride rose to 86 mEq. per liter, and he had a marked diuresis, with a weight loss of 6 kg.

CASE 6.—J. Wh., a 65-yr.-old man, entered the hospital because of dyspnea and orthopnea which had begun with an infection of the upper respiratory tract one month before and which had become progressively severer. The patient was obese, restless, and orthopneic. The blood pressure was 160/88; the temperature was 101.4 F., and the pulse rate 104. Coarse rales were heard over the emphysematous chest. The liver was slightly enlarged, but no dependent edema was present. A roentgenogram of the chest showed pulmonary congestion and moderate cardiac enlargement. The white blood cell count was 13,700 per cubic millimeter. A diagnosis of bronchopneumonia and arteriosclerotic heart disease with congestive heart failure was made.

The patient was given penicillin and was digitalized. His food and fluid intake was poor. The blood urea nitrogen on entry was 13 mg. per 100 ml. and never exceeded 26 mg. Several injections of mersalyl were given as indicated in Table 7. On the 19th day the patient showed slight mental confusion and lethargy. He was found to have a serum chloride of 69 mEq. per liter and a carbon dioxide-combining power of 31.5 mEq. Cautious use of 5% saline solution

TABLE 7.—*Summary of Clinical Data on Patient J. Wh. (Case 6)*

| | | | mEq./L. | | | Fluid Therapy | |
|---|---|---|---|---|---|---|---|
| Day | Weight, Kg. | Blood Urea Nitrogen, Mg./100 Ml. | $CO_2$ Combining Power | Chloride | Mersalyl Given, Ml. | Amount, Ml. | Solution |
| 6 | 89.6 | 13 | .... | .. | 2 | ..... | ..... |
| 7 | 84.0 | .. | .... | .. | 2 | ..... | ..... |
| 10 | 88.7 | .. | .... | .. | 2 | ..... | ..... |
| 12 | 87.2 | .. | .... | .. | 2 | ..... | ..... |
| 13 | 88.2 | .. | .... | .. | 2 | ..... | ..... |
| 15 | 87.8 | .. | .... | .. | 2 | ..... | ..... |
| 16 | 88.2 | .. | .... | .. | 2 | ..... | ..... |
| 17 | 87.2 | .. | .... | .. | .. | ..... | ..... |
| 19 | .... | .. | 31.5 | 69 | .. | 500 | 5% saline |
| 20 | 81.0 | 21 | 27.5 | 90 | .. | 250 | 5% saline |
| 21 | .... | .. | 28.5 | 84 | .. | 300 | 5% saline |
| 22 | .... | 28 | 27.0 | 90 | .. | ..... | ..... |
| 23 | .... | 35 | 24.8 | 93 | .. | 1,000 | Dextrose and saline |
| 24 | 79.5 | 36 | 22.5 | 96 | .. | 1,000 | Dextrose and saline |
| 25 | .... | .. | .... | .. | .. | 1,000 | Dextrose and saline |
| 26 | .... | 24 | 23.4 | 99 | .. | ..... | ..... |
| 27 | .... | 9 | 24.8 | 102 | .. | ..... | ..... |

corrected his salt deficiency and caused a diuresis of 6 kg. in 24 hr. No more mersalyl was given, and the electrolyte balance remained normal. Nevertheless, he became progressively more obtunded and died on his 37th hospital day. Permission for autopsy was not obtained.

## COMMENT

The observations recorded above demonstrate that therapeutic measures used to correct cardiac decompensation may have a profound influence on salt, water, and nitrogen metabolism of the body, as well as on the acid-base balance. The several patients in this report in whom ammonium chloride acidosis developed presented a fairly typical electrolyte pattern. The chloride level was always high, and the carbon dioxide-combining power and the serum pH were low. A few also showed a lowering of the sodium level. Serum potassium was usually increased but not in proportion to the rise of the urea nitrogen. With the correction of the acidosis, all elements except the potassium tended to return promptly to normal values. During the rehydration of the patient with the severest acidosis, the serum potassium showed a drop to subnormal values, a situation similar to that seen during the correction of diabetic acidosis.

Anorexia was usually the first symptom to occur in the patients who displayed ammonium chloride acidosis. It was followed by weakness, lethargy, irritability, mental confusion, and, in some cases, psychotic behavior (Cases 4 and 5). The clinical manifestations of chloride acidosis are similar to those of the salt-depletion syndrome. Restlessness and confusion have been reported in cases of dehydration following the use of mercurial diuretics.[3] Hyperpnea was usually noted with the acidosis but was not present in one severe case (Case 5). The poor hyperventilation response to acidosis may have been due to the depression of the respiratory center and its ability to respond to the stimulus of acidosis.[4]

Ammonium chloride acidosis may be confused with barbiturate poisoning. Prior to his first hospitalization, J. H. (Case 5) had taken both ammonium chloride and phenobarbital; his lethargic state was attributed to the latter drug. Later, when he was readmitted in stupor after the use of only ammonium chloride, it became apparent that both episodes of mental confusion were related to the acidosis arising from the use of ammonium chloride.

We were unable to predict which patients with uncomplicated congestive heart failure would show acidosis under our system of treatment. Symptoms were usually not observed during diuresis but developed after compensation and stabilization of weight. The presence of renal disease has long been known to hasten the development of uremia and acidosis when the plan of treatment we have described is used. Binger [5] has likewise pointed out that liver disease predisposes to the development of acidosis from ammonium chloride therapy. This fact is supported by our own experience; two of the three patients admitted in a severe state of acidosis from the use of ammonium chloride had cirrhosis of the liver. The first sign that trouble was impending was a slight rise in the blood urea nitrogen level. It frequently antedated the development of anorexia. However, the level to which the blood urea nitrogen rose did not furnish a reliable clue to the presence of acidosis. One case (not summarized) demonstrated the development of acidosis in the presence of only moderate nitrogen retention. However, those patients presenting the severest clinical manifestations of acidosis had blood urea nitrogen levels of 80 mg. per 100 ml. or higher.

Toxic symptoms and complications may be avoided by the intermittent instead of the daily use of ammonium chloride. Sartorius [6] has shown that in the first 48 hr. of ammonium chloride administration the kidneys release much sodium and potassium during the excretion of the chloride ion. He noted that by the third day, however, the normal kidney begins to excrete more ammonium ion and to conserve sodium and potassium. Because sodium and water loss are the major effects desired of a diuretic, the continual use of ammonium chloride is not as logical as its intermittent use. With this in mind, the physicians of the Cardiac Clinic of the St. Louis City Hospital have been prescribing administration of ammonium chloride

3. McCance, R. A.: Experimental Sodium Chloride Deficiency in Man, Proc. Roy. Soc., London, s.B **119**:245-268 (Feb. 1) 1936.

4. Kety, S. S.; Polis, B. D.; Nadler, C. S., and Schmidt, C. F.: The Blood Flow and Oxygen Consumption of the Human Brain in Diabetic Acidosis and Coma, J. Clin. Invest. **27**: 500-510 (July) 1948.

5. Binger, M. W., and Keith, N. M.: Effect of Diuretics in Different Types of Edema, J. A. M. A. **101**:2009-2016 (Dec. 23) 1933.

6. Sartorius, O. W.; Roemmelt, J. C., and Pitts, R. F.: The Renal Regulation of Acid-Base Balance in Man: IV. The Nature of the Renal Compensations in Ammonium Chloride Acidosis, J. Clin. Invest. **28**:423-439 (May) 1949.

for three successive days in each week. With this treatment schedule, no more cases of acidosis have been observed and satisfactory diuresis has usually been maintained.

If ammonium chloride is to be used in the management of congestive heart failure, several precautions should be observed. The development of anorexia or nausea calls for prompt discontinuance of ammonium chloride therapy before the patient is forced by his symptoms to reduce his fluid and food intake. Somnolence and mental confusion are danger signs demanding the prompt study of the blood electrolytes and the serum pH. If these determinations point to a mild acidosis or uremia, simple discontinuance of treatment with the drug is usually sufficient to correct the condition. If the acidosis is severe, oral or intravenous use of sodium lactate or sodium bicarbonate solution is indicated. The application of the formula as derived by Guest [7] is recommended.[8]

Patients receiving mercurial diuretics in the hospital are more likely to acquire salt depletion than those treated in the home, where the low-salt diet is less rigorous. Increasing weakness, pain in the calves, abdominal cramping, or a lack of satisfactory diuresis from mercurial diuretics in the presence of edema suggests the possible existence of a low serum sodium content. Serum chloride and carbon dioxide-combining power should be determined in order to give a rough estimate of the total base (sodium) present if facilities to determine the sodium are lacking.[9] It is to be remembered that acidosis and renal insufficiency may invalidate this calculation. Replacement of the sodium and chloride may be estimated on the basis of the volume of total body water or of extracellular fluid space. Peters advocates the use of the volume of total body water [10]; however, to assure ourselves a good margin of safety, we calculated the amount of electrolytes needed on the basis of volume of the extracellular space.[11] We found that we undertreated by this method and had to give small supplementary infusions of hypertonic saline solution over a two- to three-day period in some cases. Our experience with this procedure has been satisfactory; no case of pulmonary edema has occurred even when large amounts of fluids have been given.

## SUMMARY

The electrolyte patterns of 24 patients with cardiac disease have been studied before and after treatment with ammonium chloride and mercurial diuretics. In 16 of these patients serum pH studies were also made. Varying degrees of acidosis were observed, and one case of salt depletion was noted. Anorexia and drowsiness were valuable clinical signs that electrolyte imbalances were developing. The intermittent use of ammonium chloride prevented severe acidosis but allowed satisfactory diuresis.

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7. Guest, G. M.: Diabetic Coma: Metabolic Derangements and Principles for Corrective Therapy, Am. J. Med. **7**:630-646 (Nov.) 1949.

8. (*a*) Milliliters of M/6 sodium lactate solution required = body weight in kilograms × deficiency of carbon dioxide in milliequivalents × 4.2 ml., or (*b*) grams of sodium bicarbonate ($NaHCO_3$) required = body weight in kilograms × deficiency of carbon dioxide in milliequivalents × 0.058 gm.

9. Hald, P. M.; Heinsen, A. J., and Peters, J. P.: Estimation of Serum Sodium from Bicarbonate Plus Chloride, J. Clin. Invest. **26**:983-990 (Sept.) 1947.

10. Peters, J. P.: Water Balance in Health and Disease, in Diseases of Metabolism, edited by G. G. Duncan, Philadephia, W. B. Saunders Company, 1947, pp. 302-304.

11. Milliliters of 5% sodium chloride solution required = deficiency of sodium or chloride in milliequivalents × body weight in kilograms × 0.234 ml.

# Case Reports

## USE OF CORTISONE IN ERYTHEMA NODOSUM

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PITUITARY adrenocorticotropic hormone (ACTH) and cortisone (11-dehydro-17-hydroxycorticosterone) have been used successfully in treatment of many diseases due to hypersensitivity reactions.[1]

The clinical manifestations of erythema nodosum are probably the reactions of a sensitized person to a wide variety of sensitizing or antigenic substances. The sensitizing agents may be bacterial, toxic, or chemical. Erythema nodosum has been reproduced by injection of histamine [2] and antigens, such as tuberculin [3] and Streptococcus nucleoprotein.[4]

We are herewith reporting the successful treatment of a case of erythema nodosum which had been refractory to treatment for three months.

### REPORT OF CASE

M. S., a 25-yr.-old white housewife, was admitted to The Jewish Hospital of Brooklyn on Dec. 12, 1950, suffering from skin lesions on the extensor surfaces of the lower extremities.

For three months prior to admission, the patient noted the onset of purple and red areas on the legs, first on the flexor surfaces and then on the extensor surfaces. These were accompanied with "drawing" pains in the legs. No joint pains were present. There was no past history of rheumatic fever. No drugs had been taken prior to the onset of the skin lesions. Two years previously there had been occasional episodes of hemoptysis. A roentgenogram of the chest and sputum examinations at that time were said to reveal no abnormality.

She was treated with streptomycin and aureomycin by her private physician for 10 days before admission with no apparent effect. Sulfonamides had not been given.

At the time of onset of the skin lesions, she discovered that she was pregnant.

*Results of Physical Examination.*—The temperature was 99.8 F., the pulse rate, 80 per minute, and the respiratory rate, 22 per minute. The blood pressure was 108/70 mm. Hg. The patient was alert, well developed, well nourished, cooperative, and in no acute distress. The lungs were not remarkable. The heart was not enlarged. The rhythm was regular. The first apical sound was accentuated, and there was a low-pitched soft apical diastolic murmur, with presystolic accentuation. The liver, spleen, and kidneys were not palpable. The uterine fundus was enlarged to the size of a three months' gestation. No lymphadenopathy or joint tenderness was present. The extensor surfaces of both legs showed discrete and coalescing pink macular and maculopapular nodules extending up to the anterior portions of the thighs. They were slightly tender and varied from 1 to 3 cm. in diameter.

From the Department of Medicine, The Jewish Hospital of Brooklyn.

1. Bordley, J. E.; Carey, R. A.; Harvey, A. M.; Howard, J. E.; Kattus, A. A.; Newman, E. V., and Winkenwerder, W. L.: Preliminary Observations on the Effect of Adrenocorticotropic Hormone (ACTH) in Allergic Diseases, Bull. Johns Hopkins Hosp. **85**:396 (Nov.) 1949.

2. Corelli, F.: Treatment of Erythema Nodosum, Minerva med. **31**:145 (Aug. 11) 1940.

3. Wallgren, A. J.: Tuberculosis and Other Problems of Pediatrics, Abraham Flexner Lectures, series No. 10, Baltimore, Williams & Wilkins Company, 1950.

4. Spink, W. W.: Pathogenesis of Erythema Nodosum, Arch. Int. Med. **59**:65 (Jan.) 1937.

*Laboratory Data.*—The hemoglobin concentration was 13.3 gm. per 100 cc. Red blood cells numbered 4,130,000, and white blood cells, 11,250 per cubic millimeter, with 81% polymorphonuclear cells, 18% lymphocytes, and 7% eosinophils on one occasion and 2% on another. The erythrocyte sedimentation rate was 15 mm. per hour (Westergren). The Mazzini test gave a negative reaction. Antifibrinolysin was absent. Results of serial urinalyses were normal. An electrocardiogram was normal. The following blood chemical determinations were within normal limits: sugar, urea nitrogen, uric acid, creatinine, total protein, albumin, globulin, cephalin flocculation, thymol turbidity, total cholesterol, cholesterol partition, sodium, and potassium. On Jan. 2, 1951, the 24-hr. urine (1,160 cc.) contained 0.7 gm. of uric acid and 1.04 gm. of creatinine. Examination of the sternal bone marrow showed a normal condition. Roentgenograms of the chest and the bones and joints of the lower extremities were normal. A tuberculin test with 0.1 mg. of old tuberculin gave a positive reaction.

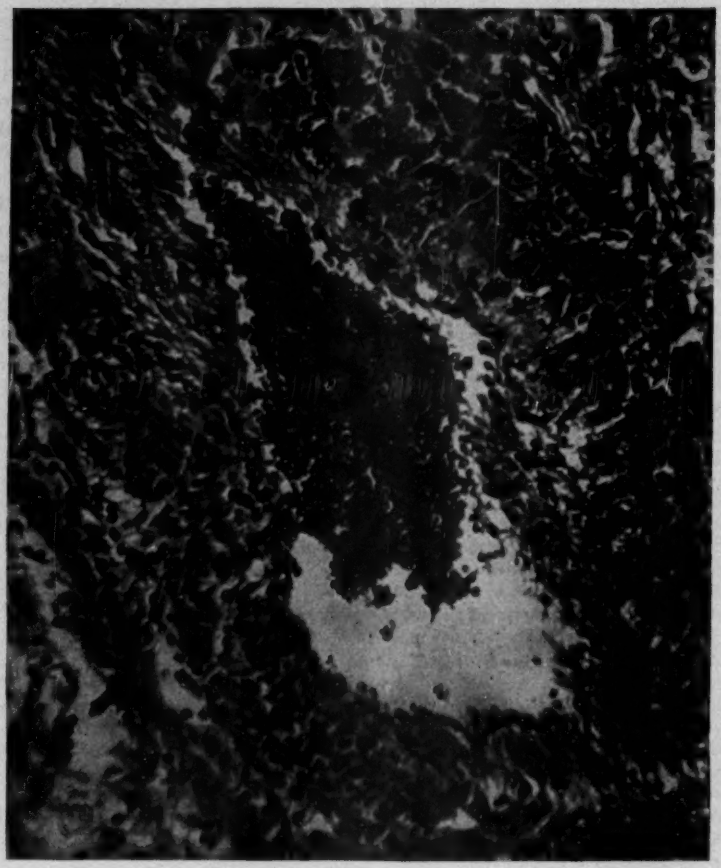

Fig. 1.—Before cortisone therapy. Photomicrograph of the cutis, showing an arteriole with intravascular erythrocytes, leucocytes, and mononuclear cells. In the surrounding tissue is a leucocyte and mononuclear infiltration (× 350).

Our clinical diagnosis was erythema nodosum. A biopsy specimen of the skin from a typical lesion (Figs. 1 and 2) showed no change in the epidermis. In the corium there was a mild perivascular reaction with edema and lymphocytic plasma-cell infiltrate. In the cutis and panniculus there were scattered foci of lymphocytes, histiocytes, and occasional eosinophils. Some arterioles and venules had polymorphonuclear cells, lymphocytes, and histiocytes in the

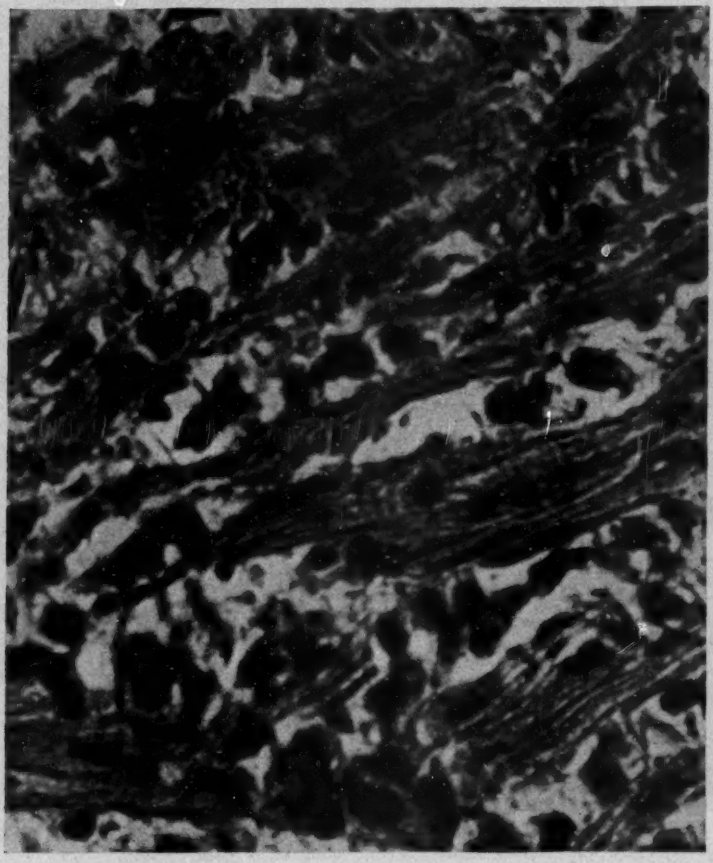

Fig. 2.—Photomicrograph of a high-power view of the wall of the arteriole, showing moderate necrobiosis with infiltration by leucocytes and mononuclear cells (× 500).

wall, with some necrobiosis. The opinion was that this was a typical appearance of erythema nodosum.[5]

*Course.*—Treatment was started with sodium salicylate (5.3 gm. per day), thiamine hydrochloride, and ascorbic acid. After a week there was no regression of the lesions. New lesions continued to appear on the legs and on the arms. *p*-Aminobenzoic acid was added, but no

5. The slides were reviewed by Dr. David M. Grayzel, of The Jewish Hospital of Brooklyn, and Dr. Paul Klemperer, of Mount Sinai Hospital, New York City, who concurred in the diagnosis.

improvement was noted. Administration of the salicylate and the *p*-aminobenzoic acid was discontinued. It was decided to use ACTH or cortisone. Eosinophil counts were done by the method of Thorn on Jan. 2 and 3, 1951. They showed 11 and 12 eosinophils per cubic centimeter. Because of the eosinopenia, it was decided to use cortisone.

On Jan. 5, 300 mg. of cortisone was given intramuscularly. By the next day, the lesions were seen to be fading and the tenderness was gone. Another 200 mg. was given. By the following day the lesions had disappeared, and on the subsequent day the cortisone therapy was discontinued. The patient had received a total of 700 mg. of cortisone. On Jan. 11 the 24-hr. urine (1,200 cc.) contained 0.062 gm. of uric acid and 1.010 gm. of creatinine.

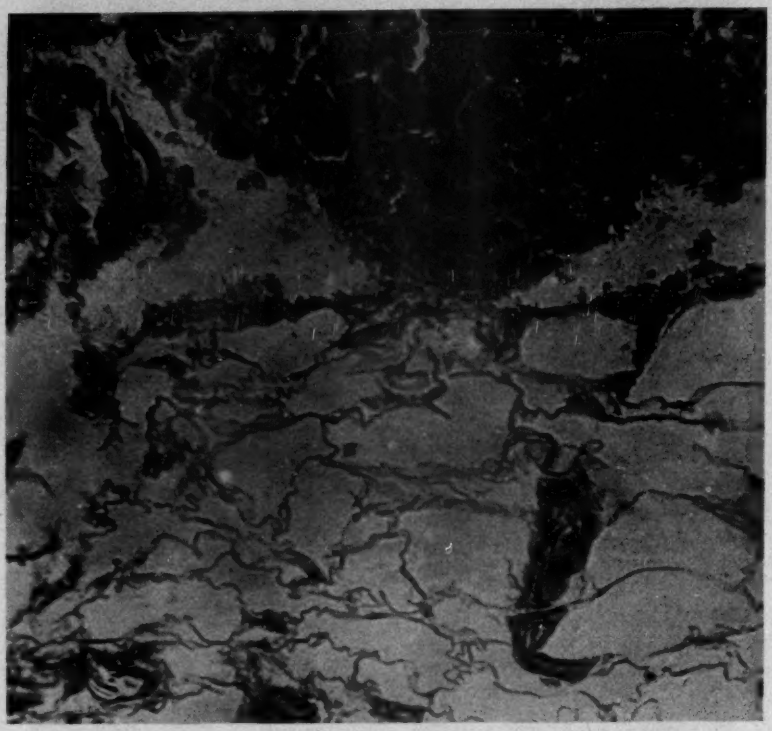

Fig. 3.—After cortisone therapy. Photomicrograph of tissue from the area where previous biopsy specimen was removed, showing normal subcutaneous tissue (× 350).

A biopsy specimen of the skin from an area adjacent to the site of removal of the first biopsy specimen, and previously demarcated as an area of erythema nodosum, was taken on Jan. 10 and showed normal skin and subcutaneous tissue (Fig. 3).

The patient was discharged without any evidence of the skin lesions present on admission.

## COMMENT

This case is unusual because of two features. Erythema nodosum generally is a self-limited syndrome and clears spontaneously in days to weeks, usually within a month. In this patient after three months there was no evidence of tendency

toward regression. It is also notable that the onset occurred shortly after the patient became pregnant, during which time an increase in the level of circulating adrenal corticosteroid would be expected. This may explain the eosinopenia. In view of the remarkable disappearance of the lesions within 48 hr. after the beginning of cortisone therapy, we may postulate that the elevated level of circulating adrenal corticosteroid simply was not adequate to prevent or reverse the reaction responsible for the appearance of erythema nodosum.

Erythema nodosum has been reported to occur in numerous conditions, among which are tuberculosis, rheumatic fever, streptococcic infections, coccidioidomycosis, drug sensitivities (sulfonamides, antipyrine, bromides, iodides), infectious exanthems, sarcoidosis, syphilis, gonorrhea, leprosy, lymphogranuloma venereum, and chancroid. Erythema nodosum may be considered a fortunate occurrence in that it is a cue for the search for one of the more serious diseases with which it could be associated. In this patient the presence of a mitral lesion which had never been noted previously was suggestive evidence of rheumatic infection. Tuberculosis had been suspected in the past, but studies were nonconfirmatory.

When thorough search fails to reveal a primary pathologic process, it is necessary to eradicate the lesions for the comfort of the patient and as a means of possibly averting a more widespread vascular disease.[6] In our patient with erythema nodosum cortisone administration proved remarkably effective as a cure.

SUMMARY

A case of erythema nodosum occurring during the first trimester of gestation is presented.

After three months without regression, there was dramatic response, evidenced by disappearance, grossly and histologically, of the lesions within 48 hr. after the institution of cortisone therapy.

6. Rich, A. R.; Berthong, M., and Bennett, I. L., Jr.: Effect of Cortisone upon Experimental Cardiovascular and Renal Lesions Produced by Anaphylactic Hypersensitivity, Bull. Johns Hopkins Hosp. **87**:549 (Dec.) 1950.

# BRONCHOBILIARY FISTULA IN HODGKIN'S DISEASE

S. TRUBOWITZ, M.D.
MONTROSE, N. Y.

THE SUDDEN appearance of deeply bile-stained sputum in the course of a disease process is indeed a striking and dramatic event. The essential dynamics for such a complication seem to be the formation of a cavity either in the substance of the liver or in a walled-off subphrenic space, but in continuity with the biliary tree, and concomitantly a mechanism maintaining an increased pressure within the bile duct to allow for a continued flow of bile through the channel between the liver and the lung. These conditions are met most frequently in the parasitic diseases of the liver, and it is especially in amebic abscesses and Echinococcus cysts of the liver that bronchobiliary fistulas may be encountered. In the nonparasitic diseases of the liver the formation of a bronchobiliary fistula is quite uncommon. This rare and, as far as I have been able to ascertain, unreported complication of Hodgkin's involvement of the liver prompted presentation of this case.

## REPORT OF CASE

A 23-yr.-old white man, A. F. (Chart 8739), was admitted to Halloran Veterans Administration Hospital in September, 1948, because of chills, fever, night sweats, and pain in the right upper abdominal quadrant which had been recurring more or less monthly since July. Sharp pain in the right side of the chest, aggravated by breathing and belching, and radiating to the right acromion, was the most prominent feature of these episodes, which would last 10 to 14 days. The pain was always accompanied with fever, chills, and anorexia. In the three months prior to admission he had lost 12 lb. (5.4 kg.) in weight. His past, as well as military, history was essentially noncontributory.

On examination he was found to be well developed, well nourished, co-operative, and intelligent and did not appear very ill. The conjunctivas were slightly injected, and there was slight erythema of the posterior pharyngeal wall. Diminished resonance was found over the lower right side of the chest and right axillary region. A soft systolic murmur was heard over the mitral area. Small, freely movable, nontender lymph nodes were present in the left axillary and inguinal regions.

The laboratory studies showed normal urine, a hemoglobin concentration of 15.4 gm. per 100 cc., a hematocrit reading (Wintrobe) of 48%, and a white-blood-cell count of 12,500, with 73% neutrophils. The thrombocytes appeared increased on smear. The Kahn reaction was negative, and a roentgenogram of the chest showed enlarged hilar nodes and elevation of the right hemidiaphragm.

It was felt on the patient's admission that he was suffering from either a malignant lymphoma or a liver abscess. An intensive and exhaustive investigation, including agglutination tests, skin tests, blood cultures, bone-marrow studies and culture, heterophile-antibody titers, and liver-function studies, was then instituted, but the results were not enlightening. However, it was observed that the white-blood-cell count was persistently elevated between 20,000 and 30,000 per cubic millimeter, with polynucleosis (85 to 90%). Fever persisted, unabated, the

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Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

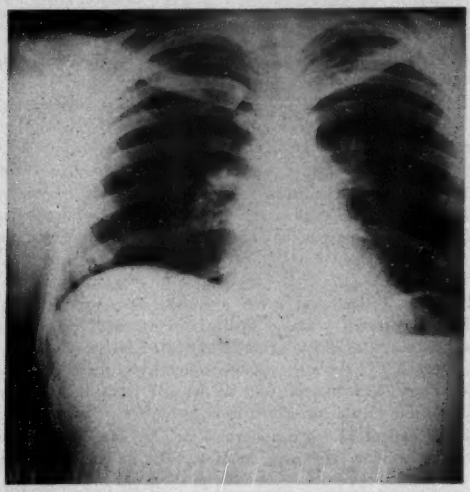


Fig. 1.—Roentgenogram of chest taken on admission, showing the high right diaphragm and the enlarged hilar nodes.

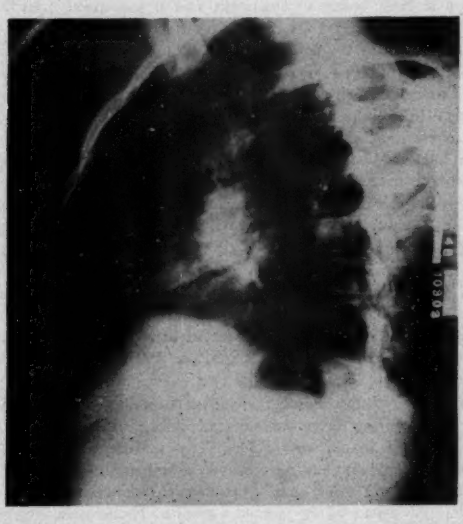


Fig. 2.—Right lateral roentgenogram of chest, showing the hilar lymphadenopathy.

temperature remaining between 99 and 103 F. Repeat roentgenographic studies of the chest showed increasing elevation of the right leaf of the diaphragm and further enlargement of the hilar densities. Pyelograms, both intravenous and retrograde, as well as gastrointestinal roentgen studies, failed to yield further information.

Stools were repeatedly searched for amebas and other parasites, without success. At no time could peripheral lymph nodes be found for biopsy.

About the middle of October, 1948, the patient had a severe nonproductive cough. At this time a friction rub was audible in the posterior axillary line of the lower right side of the chest. Treatment with penicillin and the sulfonamides was not effective. Despite negative results on stool examinations, emetine hydrochloride was administered for trial, without effect.

On Dec. 18, 1948, after two months of observation of a picture highly reminiscent of a subphrenic or hepatic abscess, an exploratory operation was performed through the resected bed of the right 12th rib. No free pus was found; instead, a diffuse granulomatous infiltration of the liver was seen. Two biopsy specimens of this material were typical of Hodgkin's granuloma.

On Dec. 21, 1948, the patient was given a course of treatment with nitrogen mustard (methyl-*bis* or *tris* [2-chloroethyl] amine hydrochloride), with prompt improvement in his condition. His cough subsided, as did the right chest pain. His temperature dropped to normal levels for the first time. X-ray therapy was then directed to the superior mediastinum and to the right upper abdomen. The patient was discharged from the hospital in March, 1949, relatively asymptomatic but with a low-grade fever and elevated right leaf of the diaphragm.

After discharge, the patient gained considerable weight. He felt well and began to work a few hours daily with no trouble. However, in May, 1949, he was readmitted because of fever, night sweats, and generalized weakness. He complained occasionally of pain in the right upper abdominal quadrant. A roentgenogram of the chest showed no change over previous films. A course of nitrogen mustard therapy was administered, with some improvement, and the patient was discharged. Between May and September, 1949, the patient felt fairly well except for night sweats and lower back pain. In September he was readmitted and observed to have enlarged right cervical nodes, a lytic lesion in the right fibula, low-grade anemia, and leucocytosis. Liver-function tests gave normal results. X-ray treatment was given to the cervical area and to the fibula, with regression of the nodes but with no apparent change in the osseous lesion.

In February, 1950, he was again readmitted to the hospital. The nonproductive cough reappeared and was associated with pain in the right upper abdominal quadrant. He had lost about 5 lb. (2.3 kg.) in weight and complained of pain in both thighs. Roentgenographic examination of the chest showed no change from previous studies. However, new involvement of both femurs was observed. He had low-grade anemia and marked leucocytosis (20,000 to 30,000 cells). Another course of nitrogen mustard therapy was administered. His symptoms subsided; the white-blood-cell count fell to normal levels. He was discharged, but the remission was short-lived. Cough, chest pain, fever, sweats, and bone pain again reappeared.

On May 29, 1950, just a few months after the last period of hospitalization, the patient was readmitted for the last time. He now had an incessant hacking cough which was productive of a small amount of thick mucus. He complained bitterly of chest and lower back pain. He appeared somewhat icteric. Moist, bubbling rales were heard in the base of the right lung posteriorly. The white-blood-cell count was elevated to 23,000, with 92% neutrophils. He had low-grade anemia. Plasma proteins were 5.2 gm. per 100 cc.; alkaline phosphatase was 55.6 units, and the serum bilirubin, 2.9 mg. per 100 cc.

During his first night in the hospital, the patient experienced violent coughing spasms and was found to be expectorating large amounts of thick, brownish, blood-tinged sputum. A diagnosis of bronchobiliary fistula was made. The sputum contained 7 to 8 mg. of bile (Harrison test) per 100 cc., and on microscopic examination fragments of skeletal muscle, necrotic material, and bacteria were seen. Numerous examinations of the sputum for amebas gave negative results. A culture of the sputum contained Escherichia intermedium and Candida (Monilia) ablicans. No tubercle bacilli were present on repeated examinations. An attempt to delineate the fistula by bronchography was not successful, but a suspicious radiotranslucent area at the termination of a bronchus was seen. Tomography did not yield anything more definitive. Bronchoscopy was deferred because of the patient's poor condition.

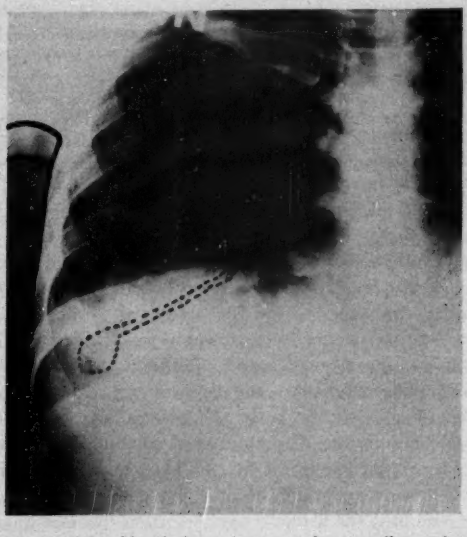


Fig. 3.—Bronchogram, right side of chest, demonstrating a radiotranslucent area below the diaphragm apparently in communication with bronchial tree which could questionably be the fistulous tract.

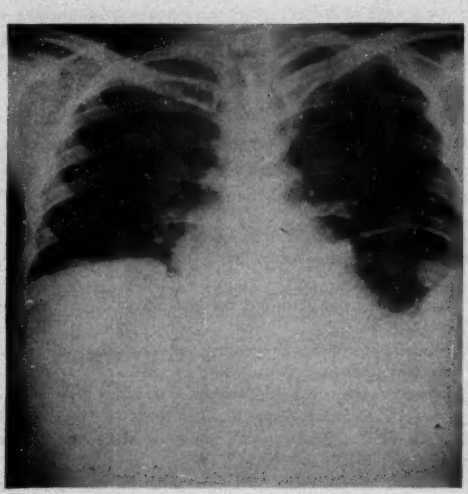


Fig. 4.—Roentgenogram of chest taken shortly before death. Note persistence of the high right leaf of the diaphragm.

During the course of the next five months, until the day of his death, the patient continued to expectorate deeply bile-stained sputum. His weight fell rapidly, and he became extremely emaciated. Jaundice became progressively deeper. The serum bilirubin finally reached a level of 7.2 mg. per 100 cc. The alkaline phosphatase remained persistently elevated at about 67 units. The white-blood-cell count ranged between 16,000 and 23,000 cells per cubic centimeter. In August, 1950, ascites and peripheral edema began to complicate the picture. Microscopic examination of the abdominal fluid showed the presence of Sternberg-Reed cells. X-ray therapy to the liver and administration of nitrogen mustard and antibiotics failed to bring about any improvement. The patient became very cachectic; multiple oral and lingual ulcers developed, and he died Oct. 8, 1950. Permission for necropsy was not granted.

## COMMENT

Bronchobiliary fistula is essentially a complication of infradiaphragmatic disease, more particularly of the liver parenchyma or extrahepatic bile ducts. Since the pulmonary lymphatic drainage is from the periphery toward the hilus, extension of a suppurative process in the lung through the diaphragm into the liver is extremely uncommon. The requisites and conditions necessary for the formation of such a fistula are to be found below the diaphragm and, according to Morton and Phillips,[1] must include an obliteration of all potential space between the liver and the diaphragm and between the diaphragm and the lung. An abscess cavity must exist either in the liver parenchyma or in a walled-off subphrenic space. The walls of the fistula must be firm and rigid, and the bile channels leading into it must be under pressure. Generally, the fistula is over the right dome of the liver, connecting with the posterior or lateral portions of the right lower lobe of the lung.[2]

Bronchobiliary fistula has been seen most frequently in association with amebic abscesses or echinococcus cysts of the liver.[3] The next commonest cause for bronchobiliary fistula may be found in biliary-tract suppuration secondary to cholelithiasis.[4] More rarely, neoplasms,[5] surgical trauma,[6] gumma, ascariasis, and tuberculosis may cause common-duct obstruction and suppuration and ultimately lead to fistula formation.

The outstanding clinical feature of bronchobiliary fistula is the expectoration of bile. In long-standing icterus small amounts of bile may be transiently found in the sputum; in fistulas there is a continuous flow of large amounts of bile.[7] Usually the complication is heralded by a critical change in the patient's condition. There is an

1. Morton, J. J., and Phillips, E. W.: Biliobronchial Fistula: Review of Recorded Cases Other Than Those Due to Echinococcus and Amebic Abscess, Arch. Surg. **16**:697-754 (March) 1928.

2. Tyramen, J.: Ein Beitrag zur Kenntnis der Gallengang-Bronchusfistel, Arch. klin. Chir. **89**:434-450, 1909.

3. Strong, R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, ed. 7, Philadelphia, The Blakiston Company, 1945, Vol. 1, p. 532. Ljubarsky, B.: Zwei Fälle von Hepatobronchial Fistulen in Folge von Echinokokkus, Arch. klin. Chir. **143**:718-726, 1926.

4. (*a*) Burgess, A. H.: Bronchobiliary Fistula, Brit. J. Surg. **9**:253-255 (Oct.) 1921. (*b*) Guy, C. C., and Oleck, H. T.: Traumatic Biliary-Bronchial Fistula: Report of 2 Cases Due to War Wounds, Arch. Surg. **55**:316-329 (Sept.) 1947.

5. Morton and Phillips.[1] Oetiker, L.: Expectoration of Bile, Schweiz. med. Wchnschr. **56**:501-502, 1926.

6. Guy and Oleck.[4b] Loe, A. O., and Loe, R. H.: Biliobronchial Fistula, S. Clin. North America **10**:1109-1117 (Oct.) 1930. French, R. W.: Biliobronchial Fistula, Arch. Surg. **30**:635-638 (April) 1935.

7. Rácz, B.: Ein Fall von Hepatobronchialfistel, Arch. klin. Chir. **172**:591-596, 1932.

intensification of the pain in the right upper abdominal quadrant, a heightening of the fever, and the appearance of a hacking, tormenting cough which finally becomes productive of bile-stained sputum. The cough is a serious and constant symptom giving the patient little rest. The quantity of sputum varies considerably and may be quite copious. Bits of tissue, stones, hooklets, or debris may be found in the sputum. The physical signs are scanty.

The cardinal roentgenological signs of bronchobiliary fistula have been listed by Flynn [8] as (1) a fixed, high right diaphragm and (2) an abscess mass near the diaphragm and involving the liver. Laird and Wilkerson [9] reported the successful instillation of iodized oil (lipiodol®) into the abscess cavity at operation. Razemon [10] established the diagnosis of a bronchobiliary fistula by the bronchoscopic injection of iodized salt. This procedure is apparently only infrequently successful.

In the cases reported in this paper, the entire symptom complex of bronchobiliary fistula as outlined above is classically duplicated. The patient's illness began with right-upper-quadrant disease which, interestingly enough, was at first highly suggestive of amebic involvement of the liver. Roentgenograms showed the fixed, high right diaphragm. An incessant hacking cough finally ushered in the diagnostic sign—the bile-stained sputum. The cause of the fistula must be attributed to the Hodgkin's involvement of the liver. The mechanism would most likely be found in some intrahepatic biliary obstruction (evidence for which may be seen in the high alkaline-phosphatase figures), secondary to Hodgkin's granuloma, with subsequent suppuration. An intrahepatic rather than extrahepatic biliary obstruction is invoked because the icterus was not pronounced, except during the last few days of life.

Necrosis in Hodgkin's disease is believed by some to be due to secondary infection and by others to interference with local circulation, and in cases of pulmonary involvement such sequelae as tracheoesophageal fistula and cavitation have been reported.[11] Although such changes have not been reported for the liver, it is reasonable to assume that in proper circumstances necrosis, cavitation, and fistula formation can take place.

SUMMARY

A case of Hodgkin's disease is reported in which the unusual complication of a bronchobiliary fistula was encountered.

Veterans Administration Hospital, Montrose, N. Y.

8. Flynn, M. J., and Warren, S. L.: Hepatobronchial Fistula, Am. J. Roentgenol. **20**:364-369 (Oct.) 1928.

9. Laird, W. R., and Wilkerson, W. V.: Biliobronchial Fistula: Report of First Recorded Case Demonstrated by Lipiodol, Am. J. Surg. **15**:317-320 (Feb.) 1932.

10. Razemon, P.; Bizard, G., and Lambret, M.: Les fistules bronchobiliaires consécutives à la lithiase, Rev. chir., Paris **52**:485-505 (July) 1933.

11. Wallhouser, A.: Hodgkin's Disease, Arch. Path. **16**:522-562 (Oct); 672-712 (Nov.) 1933. Jackson, H., and Parker, F., Jr.: Hodgkin's Disease and Allied Disorders, New York, Oxford University Press, 1947.

# Clinical Notes

## THE DIVIDED CULTURE PLATE

### Its Use in Testing for Sensitivity to Antibiotics

JOSEPH FELSEN, M.D.
AND
ALFRED J. WEIL, M.D.
NEW YORK

The divided culture plate[1] was devised principally as a practical aid in the bacteriologic diagnosis of enteric infection and for antibiotic studies. It consists of the standard 94-mm. Petri-dish bottom, which is separated into quarters by two smooth bisecting ridges of glass 5.5 mm. in height. The inside height of the bottom is 13 to 15 mm., and the covers are identical with those of ordinary Petri dishes. The quadrants are marked I to IV for easy identification. In studies on enteric pathogens, the suspected material (feces or, preferably, exudate obtained by mucosal crypt aspiration[2]) is seeded on the quadrants, each of which contains a different culture medium chosen for its selectivity (e. g., Endo's [fuchsin sulfite agar], S. S. [Salmonella-Shigella thiosulfate-citrate bile agar], desoxycholate citrate agar, and MacConkey's [bile-salt agar] or E. M. B. [eosin methylthionine chloride agar]). The plates are incubated overnight and suspicious colonies picked for rapid serologic identification by slide or tube method. After two years of use in this field, the plates have proved most informative and economical. The advantages of four differential mediums on a single plate are easier reading and the saving of three Petri dishes. This economy of time, material, and effort is noteworthy for both military and civilian practice.

In studies on antibiotic sensitivity, we make use of antibiotic-impregnated paper disks.[3] All except aureomycin come in three strengths.[4] These are as follows:

| | |
|---|---|
| Penicillin | 0.5, 1.0, 10 units |
| Chloramphenicol | 10, 30, 60 micrograms |
| Terramycin | 10, 30, 60 micrograms |
| Streptomycin | 0.5, 1.0, 10 micrograms |
| Dihydrostreptomycin | 0.5, 1.0, 10 micrograms |
| Bacitracin | 2, 10, 20 units |

Aureomycin-impregnated disks are available in one strength, 60 micrograms.[5] Each antibiotic has a distinctive color. The plates are seeded with the organism isolated from the blood,

From the Department of Laboratories and Medical Research, The Bronx Hospital.

1. Felsen, J.: Divided Culture Plate, Am. J. Clin. Path. **19**:289-290 (March) 1949.

2. Felsen, J.: Crypt-Aspiration Spray Culture Method for the Isolation of B. Dysenteriae, J. Lab. & Clin. Med. **23**:630-632 (March) 1938.

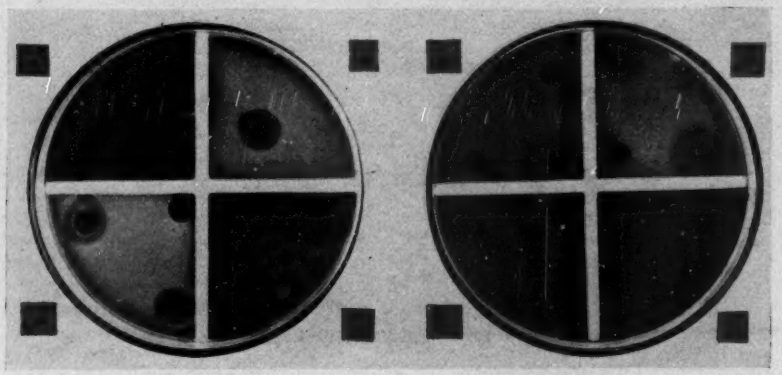
3. Vincent, J. G., and Vincent, H. W.: Filter Paper Disk Modification of the Oxford Cup Penicillin Determination, Proc. Soc. Exper. Biol. & Med. **55**:162-164 (March) 1944. Loo, Y. H.; Skell, P. S.; Thornberry, H. H.; McGuire, J. M.; Savage, G. M., and Sylvester, J. C.: Assay of Streptomycin by the Paper-Disc Plate Method, J. Bact. **50**:701-709 (Dec.) 1945. Bondi, A., Jr.; Spaulding, E. H.; Smith, D. E., and Dietz, C. C.: A Routine Method for the Rapid Determination of Susceptibility to Penicillin and Other Antibiotics, Am. J. M. Sc. **213**:221-225 (Feb.) 1947. Thompson, B. A.: Dried Disk Technique for Determining Sensitivity to the Antibiotics, J. Clin. Path. **3**:118-124 (May) 1950.

4. The antibiotic-impregnated paper disks (except aureomycin) are available from Difco Laboratories, Detroit. Antibiotic-testing tablets are available from Boyle & Company, Los Angeles.

5. Aureomycin-impregnated paper disks are available from Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

abscess, or other site of infection, and the three disks of a given antibiotic are placed in the corners of each quadrant, as illustrated. By using the apex and left and right corners, in the order mentioned, the strengths of the corresponding disks can be identified. Two divided culture plates are used, one quadrant being allocated to each antibiotic. The eighth quadrant is used as a control. The plates are incubated overnight, and the clear zones indicating inhibition of growth, and therefore sensitivity of the organism to the antibiotic, are measured. The antibiotic exhibiting the greatest inhibition of growth is selected for therapy.

In general, there is a close correlation between the in-vitro and in-vivo action of antibiotics. In clinical work, the usual practice has been to try one antibiotic after another until one is found to be effective. Much valuable time may be lost, often to the detriment of the patient. This is illustrated by a few examples from our hospital. In three newborn infants with empyema, one with bacteremia and osteomyelitis and two with large abscesses, the infecting organism was found to be a hemolytic coagulase-positive Staphylococus pyogenes var. aureus which was extremely resistant to penicillin but sensitive to aureomycin.[6] A child with Hemophilus influenzae meningitis received the accepted streptomycin therapy until we found the organism to be insensitive to it, but highly sensitive to aureomycin, chloramphenicol (chloromycetin®), and terramycin. In another child, Staph. pyogenes var. aureus, Staphylococcus



Antibiotic-sensitivity test with Streptococcus faecalis hemolyticus isolated from a patient with empyema of the gall bladder. Blood agar plates show zones of inhibition, represented by circular areas around disks, and intervening areas of hemolysis and bacterial growth: (1) penicillin, (2) aureomycin, (3) chloramphenicol, (4) terramycin, (5) streptomycin, (6) dihydrostreptomycin, (7) bacitracin, and (8) growth control.

pyogenes var. albus, and alpha hemolytic Streptococcus were recovered from a breast abscess. All three organisms were sensitive to chloramphenicol and showed little sensitivity to the other antibiotics. In the few examples cited an immediate change in therapy to the indicated antibiotic produced satisfactory results. There is an increasing number of reports of primary strains which are resistant to the usually effective antibiotics.[7]

It takes approximately 18 hr. for the sensitivity tests to be completed after the primary seeding or after the organism has been isolated. The procedure tends to eliminate the element of guesswork in antibiotic therapy and is extremely simple to carry out. During the delay in

6. Finland, M.; Frank, P. F., and Wilcox, C.: In Vitro Susceptibility of Pathogenic Staphylococci to Seven Antibiotics (Penicillin, Streptomycin, Bacitracin, Polymyxin, Aerosporin, Aureomycin, and Chloromycetin), Am. J. Clin. Path. **20**:325-334 (April) 1950.

7. Finland and others.[6] Felsen, J.; Lapin, J.; Wolarsky, W.; Weil, A. J., and Fox, I.: Staphylococcic Infections in Hospital Nurseries and Pediatrics Wards, A. M. A. Am. J. Dis. Child. **81**:534-540 (April) 1951.

isolating the organism, an antibiotic of wide antibacterial pattern may be used if the patient's condition is critical, or the choice may be made based upon the clinical diagnosis and the known effectiveness of a given antibiotic in the disease diagnosed. In urgent cases, one may minimize delay, even with mixed cultures, by setting up the unknown against the various antibiotics without waiting for pure cultures. This means presentation of a fairly clear directive to the clinician within about 18 hr. after primary growth has been obtained. Further definitive studies are then made for greater accuracy.

The tests as outlined can be carried out without any undue strain upon the resources of the bacteriologic laboratory. There is a reasonable parallelism between the results of the disk-divided culture plate and standard dilution methods. Another significant advantage of the disk method lies in the detection of resistant mutants, which appear as isolated colonies within the zone of inhibition. If these are present, the clinician is advised of probable impending resistance to the antibiotic in question.

# News and Comment

## ANNOUNCEMENTS

**Essay Award.**—The Board of Regents of the American College of Chest Physicians offers a cash prize award of $250 to be given annually for the best contribution, preferably by a young investigator, on any phase relating to chest disease. The prize is open to contestants of other countries, as well as those residing in the United States. The winning contribution will be selected by a board of impartial judges, and the award, together with a certificate of merit, will be made at the forthcoming annual meeting of the College. Second and third prize certificates will also be awarded.

All manuscripts submitted become the property of the American College of Chest Physicians and will be referred to the Editorial Board of the College journal, *Diseases of the Chest*, for consideration. The College reserves the right to invite the winner to present his contribution at the annual meeting. Contestants are advised to study the format of *Diseases of the Chest* as to length, form, and arrangement of illustrations, to guide them in the preparation of the manuscript.

The following conditions must be observed: 1. Five copies of the manuscript, typewritten in English, should be submitted to the executive office, American College of Chest Physicians, 112 E. Chestnut, St., Chicago 11, not later than April 1, 1952. 2. The only means of identification of the author or authors shall be a motto or other device on the title page, and a sealed envelope bearing the same motto on the outside, enclosing the name of the author or authors, should be included.

**Sixteenth Annual Convention of National Gastroenterological Association.**—The National Gastroenterological Association will hold its Sixteenth Annual Convention and Scientific Sessions at The Drake Hotel, in Chicago, on Sept. 17, 18, and 19, 1951.

The program will include a Symposium on Peptic Ulcer in which Dr. Andrew C. Ivy, of the University of Illinois, will present the Physiological Aspects; Dr. David J. Sandweiss, of Detroit, the Medical Aspects, and Dr. Lester R. Dragstedt, of Chicago, the Surgical Aspects.

There will also be a Symposium on the Pancreas, a Symposium on Psychosomatic Medicine, and a Symposium on Carcinoma, and other individual papers.

Other speakers include Dr. C. Wilmer Wirts, Philadelphia, Dr. C. P. Rhoads, New York, Dr. R. Russell Best, Omaha, Neb., Dr. Garfield G. Duncan, Philadelphia, Dr. Sidney A. Portis, Chicago, Dr. Walter L. Palmer, Chicago, and Dr. Fred J. Hodges, Ann Arbor, Mich.

The convocation ceremony, at which certificates will be presented to the newly elected and advanced Fellows and Associate Fellows and newly elected Members will take place at The Drake on Monday afternoon, Sept. 17.

This meeting will see the inauguration of a new idea in commercial exhibits. The Association is limiting its exhibit space to those concerns which will present a scientific type of exhibit and will have members of their scientific and research staff present at the meeting.

In addition, a luncheon for those attending will promote closer understanding between the representatives of the pharmaceutical concerns and the physicians.

Immediately following the convention, on Sept. 20, 21, and 22, 1951, the Association is conducting a Course in Postgraduate Gastroenterology at The Drake under the direction of Dr. O. H. Wangensteen, Minneapolis, and Dr. I. Snapper, New York.

Further information concerning the program and details of the course may be obtained by writing to the Secretary, National Gastroenterological Association, 1819 Broadway, New York 23.

**Motion Picture.**—A revised catalog of motion pictures available through the Committee on Medical Motion Pictures of the American Medical Association is now available. Copies will be sent to the secretary of each county and state medical society. This catalog lists 62 16-mm. films, most of which are at the professional level. Fourteen of these films are suitable for showing to lay groups. Eight new films have been added. Copies are available upon request from the Committee on Medical Motion Pictures, American Medical Association, 535 N. Dearborn St., Chicago 10.

**Annual Postgraduate Course in Diseases of the Chest.**—The annual postgraduate course in diseases of the chest sponsored by the Council on Postgraduate Medical Education and the New York State Chapter of the American College of Chest Physicians, will be presented at the Hotel New Yorker, New York, Nov. 12 to 17, 1951.

This course will emphasize the recent advancements in the diagnosis and treatment of chest diseases. The course is open to all physicians, but the number of registrants will be limited. Tuition fee is $50; applications will be accepted in the order in which they are received. Applications should be sent to the American College of Chest Physicians, 112 East Chestnut Street, Chicago 11.

**Postgraduate Courses Arranged by the American College of Physicians.**—The American College of Physicians has announced a series of postgraduate courses to be held in the autumn of 1951. These include "Internal Medicine: Selected Subjects" (Oct. 8 to 13, 1951); "The Diagnosis and Treatment of Cardiovascular Disease" (Oct. 22 to 27, 1951); "Clinical Neurology" (Oct. 29 to Nov. 2, 1951); "Electrocardiography" (Oct. 29 to Nov. 3, 1951); "Gastro-Enterology" (Nov. 12 to 17, 1951); "Recent Advances in the Diagnosis and Treatment of Cardiovascular Disease" (Nov. 12 to 17, 1951); "Cardiovascular Diseases" (Dec. 10 to 15, 1951), and "Physiological Basis for Internal Medicine" (Dec. 10 to 15, 1951). Further information concerning these courses may be secured from Mr. E. R. Loveland, The American College of Physicians, 4200 Pine St., Philadelphia 4.

## GENERAL NEWS

**Grant to Aid Cerebral Palsy Education.**—A grant of $10,000 to continue a nationwide educational program for specialists in the field of cerebral palsy has been made by Alpha Chi Omega, woman's fraternity, to the National Society for Crippled Children and Adults, Chicago, the Easter Seal Agency.

The grant was voted at the fraternity's 28th annual convention in Roanoke, Va., and will make possible advanced study in the care and treatment of cerebral-palsied children by specially selected physicians, therapists, and educators during the next two years.

It brings to $35,000 the total grants made by Alpha Chi Omega to the National Society for training on the national level. In addition, $55,000 has been given for scholarships and training on the local level.

Thus far, scholarship winners have been selected from 36 states, Hawaii, Canada, and Denmark, according to the announcement by Lawrence J. Linck, executive director of the National Society. Since the beginning of the project, 54 physicians, therapists, and educators have received special training as a result of the Alpha Chi Omega grants.

## NOTICES

**Symposium on Rheumatic Fever.**—The University of Minnesota, Minneapolis, announces a Symposium on Rheumatic Fever to be presented Nov. 29, 30, and Dec. 1, 1951. The Symposium is being given with the sponsorship and financial support of the Minnesota Heart Association. Distinguished medical scientists who have accepted an invitation to participate include Dr. T.

Dunkett Jones, Dr. Ann Kuttner, Dr. Maclyn McCarty, and Dr. George Murphy, of New York; Dr. Francis Schwentker, Baltimore; Dr. Chandler A. Stetson, Cheyenne, Wyo.; Dr. Charles Rammelkamp, Cleveland, and Dr. Lowell Rantz, San Francisco. Members of the faculty of the University of Minnesota Medical School and of the Mayo Foundation will also participate. All interested physicians, teachers, and investigators are cordially invited to attend. There will be no tuition or registration fees.

## PUBLICATIONS

**Booklet on Immunization for Travelers.**—The latest facts on immunization for travelers going to every section of the world are detailed in a booklet just released by the United States Public Health Service. The title of the booklet is "Immunization Information for International Travel." It includes official information on the immunizations required and recommended by each country and the immunizations recommended by the Public Health Service as a precautionary measure for persons traveling abroad. Other items of importance to the traveler include an explanation of the procedure for having inoculations recorded on the International Certificate of Inoculation and Vaccination; a list of Public Health Service facilities where yellow fever inoculations can be obtained, and maps showing the yellow fever endemic areas of the world.

All changes in immunization requirements made after the publication of this booklet will be given in the weekly "Communicable Disease Summary," released by the Public Health Service, under the heading "Quarantine Measures." Travelers can obtain this information from local and state health departments.

The booklet may be purchased from the Superintendent of Documents, Government Printing Office, Washington, D. C., for 20¢ a copy. A 25% discount is allowed on orders of 100 copies or more delivered to the same address.

# Book Reviews

**Medical Diagnosis: Applied Physical Diagnosis.** Edited by Roscoe L. Pullen, M.D., F.A.C.P. Price, $12.50. Pp. 1119, with 601 illustrations, 48 in color. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1950.

The second edition of this volume on applied physical diagnosis is written by 23 contributors, each of whom is outstanding in his particular field of endeavor. The book consists of 24 chapters developed along a regional approach. Laboratory procedures and roentgenography, which have become so increasingly important from a diagnostic standpoint, are correlated very well with the techniques of physical examination of the various regions. There are new chapters devoted to the blood, the aged, and the physical examination of the psychiatric patient. The section on electrocardiographic diagnosis is especially commendable, but it is doubtful that such a specialized subject should occupy the same amount of space as the physical examination of the heart. Other minor criticisms are the seemingly excessive number of pages devoted to oral diagnosis and gynecologic and obstetric diagnosis.

There is an excellent cross-index, and the book is printed in an easily readable form. In spite of its considerable size this book will find much favor with the student, and it seems destined to become the standard reference on applied physical diagnosis.

**Wege vergleichender Terapie in der inneren Medizin: Zugleich ein Bitrag zur Klinik der Lungenentzündung, Ruhr, Nierenentzündung und Genickstarre: I. Einführung. II. Lungenentzündung.** By Robert E. Mark. Price, 26 German marks. Pp. 418, with 127 illustrations. Urban & Schwarzenberg, Meinekestr. 13, Berlin W. 15, 1950.

The contents of this book are divided into two parts. The first is concerned with an introduction to comparative therapy. Under this heading the author discusses the reasons that one must continually practice comparative therapy, and then he outlines the various methods of accomplishing this.

The second main division presents observations on pneumonitis in the light of comparative therapy. In general, the author concerns himself with lobar pneumonia of pneumococcic origin and nonviral bronchopneumonias. He studied the effect of purely symptomatic treatment and calcium quinine and sulfathiazole therapy on these pneumonias and concludes that, of the therapeutic measures, sulfathiazole administration is by far the most efficacious. The methods used are of interest, particularly to the medical statistician, and the importance of comparative therapy is certainly worthy of the physician's attention. If this had been borne in mind, certainly many persons would have been spared the hope and pain which accompanied the use of the so-called cold vaccine.

The volume is paper-bound and poorly presented, and the practicing physician can use his time to better advantage than in reading this book.

**Zur Frage der Cortison-Wirkung (die "Corticogene Kettenreaktion").** By Prof. Dr. W. Beiglböck, Dr. H. Hoff, and Dr. R. Clotten. Price, 6.50 marks. Pp. 142, with charts. Hans Höfle, Fuchsweg 13, Augsburg II/land, (13b) Inningen, 1950.

These authors have not used cortisone. They reviewed the literature and compiled a mass of speculation without the addition of anything new. The book is of doubtful value and may lead readers not engaged in research in this field astray because of the authoritative way in which it is written. The review of literature is not critical; apparently, one set of results is regarded as being of the same value as another merely because they both appeared in print.

**Physiologie und Pathologie des Bilirubinstoffwechsels als Grundlagen der Ikterusforschung.** Dr. Med. et Phil. Habil. By T. Baumgärtel. Price, 27 German marks. Pp. 271, with 2 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A.: Grune & Stratton, Inc., 381 4th Ave., New York 16, 1950.

This monograph is mainly concerned with an inquiry into the causes of icterus. After a fairly lengthy review of the historical development of the icterus problem, the author divides his

subject material into two main divisions: (1) the physiology of bilirubin metabolism and (2) the pathology of bilirubin metabolism. The author is well equipped to undertake this study, since he was a student and co-worker of Dr. Hans Fischer. This work is not designed to furnish bedside help in the study of the patient with jaundice, but, because of its detailed inquiry into the chemistry and physiology of bilirubin, it makes an excellent reference work. The author has covered the literature thoroughly, and the references are located at the bottom of the pages so that they can be readily found. Because of the many references, the text is a little difficult to read. This monograph may be recommended to anyone interested in the metabolism of bilirubin, particularly for the presentation of the author's original work, as well as for the literature on this subject.

**Perspectives in Human Malnutrition.** By Joseph Gillman. D.Sc., M.B., B.Ch., and Theodore Gillman, M.Sc., M.B., B.Ch. Price, $18. Pp. 608, with 260 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1951.

This is an interesting and timely book. Its authors live and work in South Africa and clearly have had opportunity to observe malnutrition in that part of the world and to compare its manifestations in native Africans with its effects on Europeans who have moved there.

Chief emphasis is placed on pellagra, but other states of malnutrition are considered, especially nutritional edema. The entire subject of malnutrition is discussed well and intelligently, for example, its effect on the alimentary tract, on the liver and pancreas, on bones, on the endocrine glands, and on the nervous system.

The volume is profusely illustrated with charts, diagrams, and well-reproduced photographs. A comprehensive bibliography is appended, so that almost every significant contribution to the knowledge of malnutrition is woven into the text.

It is a long, complete book, and in order to save bulk, presumably, a two-column format has been utilized. On the whole, it represents a notable piece of work. Certainly all medical libraries will wish to have a copy, as will all physicians with serious interest in the absorption, digestion, and utilization of food.

**Chronic Ulcerative Colitis (Thrombo-Ulcerative Colitis).** By Jacob Arnold Bargen, M.D. Price, $2. Pp. 62, with 3 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

This brief booklet is another publication of the American Lecture Series. Dr. Bargen covers the subject in standard fashion, and the discussions of debatable points are sound and well tempered. Nothing is said about cortisone or corticotrophin (ACTH), and perhaps this is not unreasonable until more carefully controlled studies have been made.

**Stoffwechsel und Ernährung.** By Dr. Konrad Lang and Dr. Otto F. Ranke. Price, 19.80 marks. Pp. 289, with 154 tables. Springer-Verlag, Reichpietschufer 20, Berlin W. 35, 1950.

The authors provide in this volume a critical exposition of experimental exploration and practical experience in human nutrition. The early chapters deal with the principles of metabolism, the methods available for determining energy balance, basal metabolism, and energy requirements for work, and the effects on metabolism of semistarvation and starvation. Later topics covered include utilization of food and the significance in nutrition of fats, carbohydrates, proteins, minerals, and vitamins. The concluding chapter covers the effects of malnutrition on the liver, on reproduction, and on longevity. The book ends with the importance of bread and its components in the human economy, of certain newer protein foods, and of vegetarianism.

The book is liberally documented and provides for the reader of German texts an excellent introduction to this field of knowledge, a field which in the present insecure state of world affairs is of utmost importance to human welfare.

**Allgemeine Pathologie.** By Franz Buchner. Price, 32.80 German marks. Pp. 528, with 424 illustrations. Urban & Schwarzenberg, Meinekestr. 13, Berlin W. 15, 1950.

This volume, although entitled "General Pathology," is actually an attempt to correlate disturbed physiologic changes with pathology. The book is largely the result of the author's lectures at the Pathological Institute of the University of Freiburg im Breisgau. The author

indicates by a subtitle that his book is concerned with pathology as biology and is a treatise on the study of man. In order to accomplish his ends the author divides the book into the following main divisions: (1) an introduction in which he discusses the problem of the living: the fundamental phenomena of life and a history of the philosophy of the living; this introduction further concerns itself with health and disease and the position of man in the animal kingdom; (2) the general pathology of the elementary structure; (3) the general pathology of the experiences of life; under this heading the author covers a myriad of subjects, dealing in a cursory fashion with, for example, the physiology of metabolism, the physiology of the porphyrins, the pathology of digestion, the general pathology of breathing, etc.; (4) the general pathology of heredity and environment. In this category he discusses the pathologic effect of warmth and cold and the importance of the weather in pathology. The final important division concerns itself with the pathogenic significance of psychosomatic factors.

At best, the author can hope to treat most of his subjects in only a superficial fashion. It is a little disturbing when one finds in a textbook by the Director of the Ludwig Aschoff House of the Pathological Institute of Freiburg only a few paragraphs on rheumatic heart disease and only a few pages on the effect of weather on pathology. One must commend the author, however, on his desire to correlate as nearly as possible the problems of the practice of medicine and pathology. He places great emphasis on "the strength of the physician's sustaining love for his patients," and he continually emphasizes the importance of considering the individual when one studies the various pathologic manifestations of disease process.

The volume is well bound; the paper is excellent, and the type and figures leave little to be wished.

**System of Clinical Medicine: Dealing with the Diagnosis, Prognosis and Treatment of Disease for Students and Practitioners.** By Thomas Dixon Savill. Thirteenth edition. Edited by E. C. Warner, M.D., F.R.C.P. Price, $7. Pp. 1,198, with 195 illustrations and 7 colored plates. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1950.

The thirteenth edition of this old standby is in much the same format as the previous volumes. As every one knows, Dr. Savill's system was to work from the principal symptoms on to the correct diagnosis. Hence the material is arranged somewhat differently from the usual textbook style. There is much useful information between these covers, even though no "system" or "short cut" will provide an easy road to secure diagnosis without experience and fundamental training.

**Therapeutics in Internal Medicine.** Edited by Franklin A. Kyser, M.D., F.A.C.P. Price, $12. Pp. 715, with illustrations. Thomas Nelson & Sons, 385 Madison Ave., New York 17, 1950.

This handy volume actually contains some 700 pages double spaced and represents an excellent up-to-date summary of treatment in internal medicine. Each disease is dealt with in a brief separate article signed by the individual author. One feels the impersonality of a composite book of this sort, but it seems impossible to present the material in any other way nowadays, so that one has to sacrifice for the sake of efficiency the development of an individual point of view of the whole subject.

**Toxaemias of Pregnancy, Human and Veterinary: A Ciba Foundation Symposium.** Edited by John Hammond, D.Sc., F.R.S.; F. J. Browne, M.D., D.Sc., F.R.C.S., F.R.C.O.G., and G. E. W. Wolstenholme, O.B.E., M.B. Price, $4.50. Pp. 280, with 93 illustrations. Publisher: The Blakiston Company (Division of Doubleday & Company, Inc.), 1012 Walnut St., Philadelphia 5, 1950.

This book contains a series of lectures on the toxemias of pregnancy, given under the auspices of the Ciba Foundation. Following a page of introduction by Lord Horder are some two dozen papers on various phases of toxemia of pregnancy, human and animal. The authors come from many countries, and their various points of view are not to be briefly reviewed. The medical and obstetrical summaries by Pickering and by Browne, which conclude the volume, are especially useful.

**Pharmacological Basis of Penicillin Therapy.** By Karl H. Beyer, Ph.D., M.D., F.A.C.P. Price, $4.50. Pp. 214, with 37 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1950.

This little book contains a comprehensive review of the pharmacology of penicillin. The author's interest in the tubular blocking of penicillin excretion takes him over into the pharmacology of carinamide, which is certainly of interest, although the edge has been taken off the clinical use of blocking substances since slowly absorbed preparations of penicillin have been devised.

**Lehrbuch der Inneren Medizin. Volume II.** Edited by Helmut Dennig. Price, 39 German marks. Pp. 1,117, with illustrations. Georg Thieme, Diemershaldenstrasse 47, Stuttgart, Germany; agents for the United States: Grune & Stratton, Inc., 381 4th Ave., New York 16, 1950.

This is an excellent book and covers in a comprehensive fashion diseases of the heart and blood vessels, diseases of the digestive system, diseases of the nervous system, allergy and poisons; a general consideration of diagnosis and treatment of internal diseases concludes the work.

This second volume is edited by Dr. H. Dennig, of Stuttgart, and he has as collaborators nine other professors of internal medicine in Germany, which should reflect fairly well current medical practice in that country.

The section on diseases of the nervous system is to be especially emphasized, as it is extremely well done and colored charts assist the reader in obtaining a clearer picture of the various abnormalities. The concluding chapter deals with the bedside application of the treatment and diagnosis of internal diseases. This approach is now frequently seen in German texts, and the reviewer feels that our American textbooks on medicine would profit by such chapters. This volume is to be recommended.

**Pulmonary Ventilation and Its Physiological Regulation.** By John S. Gray, M.D., Ph.D. Price, $2. Pp. 82, with 14 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1950.

Gray's synthesis of the seemingly divergent facts regarding the physiology of pulmonary ventilation is a strong case for the essentially cooperative nature of "independent" physiological regulatory systems. The development of the "multiple factor" theory, especially in response to the impetus given to pulmonary research in aviation medicine during World War II, has been interesting to observe and gratifying in its clarification of erstwhile paradoxes. Current physiological literature reflects the insight which may be gained into this clinically important field by the application of this approach. Admittedly not all phenomena of pulmonary regulation are known, and some now observed are not incorporated into the present integral devised by Dr. Gray, but it seems evident that a sound basic principle has been established on which further elaborations can be made. The book is clearly written and should be read by all research and clinical workers in this field. It may well point the way in laying the groundwork for understanding of the regulation of other organ systems.

**Immunology.** Third edition. By Noble Pierce Sherwood. Price, $8. Pp. 731, with many illustrations. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1951.

This is a successful textbook on immunology. The first edition appeared in 1935, the second in 1941, and the third has now been recently printed. All three have followed the same general pattern but have grown in size to keep up with developments in knowledge.

The ARCHIVES reviewed the first edition (**58**:956 [Nov.] 1936), commenting favorably on the arrangement of the text, the bibliography that appeared at the end of each chapter, and the excellent index. The same compliments can be paid the third edition. Indeed, the words that concluded the review of the first edition still hold true in regard to the third. Again it can be said that "the book belongs to that attractive species which at once feels at home and is a useful member of the family in any library, on any reference shelf or in any laboratory."

**Paul Ehrlich.** By Martha Marquardt. With an introduction by Sir Henry Dale. Price, $3.50. Pp. 255, with illustrations. Henry Schuman, Inc., Publishers, 20 E. 70th St., New York 21, 1951.

Miss Marquardt presents a vivid picture of one of the great scientists of all time. While Ehrlich's work is dealt with in considerable detail, the author's idea has been also to expose the nature of the man and to portary him as a real person. This line is skilfully followed, and the device of reporting "conversations" between Ehrlich and his friends and colleagues makes everything very realistic. On the whole, however, the book is essentially one of appreciation, but this is no criticism, since surely this great man, like others, deserves his Boswell.

There are numerous interesting photographs of both persons and pertinent places.

**Handbook on Diseases of Children, Including Diabetics and the Common Fevers.** Sixth edition. By Norman Bruce Williamson, M.D. (Edin.), F.R.C.P. (Lond.). Price, $4.50. Pp. 440, with 94 illustrations and tables. Williams & Wilkins Company, Mount Royal & Guilford Aves., Baltimore 2, 1951.

This book, well printed on good paper, with many excellent photographs and charts, is "boiled down" almost to pocket size. However, wherever the reviewer dipped into it he found the subject under discussion dealt with in up-to-date and sound fashion. British medicine, on the whole, is somewhat more conservative than American medicine, and one finds more stress on clinical features, rather than on elaborate chemical measurements, than would be likely in a textbook of pediatrics published in this country.

**Symposium on the Healthy Personality: Transactions of Special Meetings of the Conference on Problems of Infancy and Childhood, June 8-9 and July 3-4, 1950, New York.** Edited by Milton J. E. Senn, M.D. Price, $2.50. Pp. 298. Josiah Macy, Jr., Foundation, 565 Park Ave., New York 21, 1950.

The Macy Foundation has done pediatricians and psychologists a great service in sponsoring this "Symposium on the Healthy Personality." The deliberations of a panel of experts are set forth in a handsome book of 298 pages dealing with growth and crises, constitutional and prenatal factors and the social psychology of mental health. The discussion is of great interest but does not lend itself to detailed review.

**An Introduction to Universal Serologic Reaction in Health and Disease.** By Reuben L. Kahn, D.Sc. Price, $3.50. Pp. 155, with 113 charts. Commonwealth Fund, Division of Publications, 41 E. 57th St., New York 22, 1951.

Dr. Kahn, long an authority on the serology of syphilis, now presents a book dealing with serologic reactions in more general fashion. The subject is a highly technical one, and this compendium will certainly be of utmost value to special workers in the field. More particularly, Dr. Kahn deals with the value of lipid antigens and points out their usefulness in various diseases other than syphilis, such as leprosy, malaria and tuberculosis.

**Virus and Rickettsial Diseases.** By S. P. Bedson, M.D., and others. Price, $5.50. Pp. 383, with 33 illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1951.

This compact text ably fulfils the purpose set forth by its authors in the preface, that is, to provide an authoritative, compact reference to the virus and rickettsial diseases for the practicing physician. It presents in concise, exact language a summary of the newer knowledge in regard to these maladies. In addition, the reader is provide with an excellent bibliography at the end of each chapter.

The introductory sections are especially well written, particularly the ones on immunity and chemotherapy.

Considering the fact that chloramphenicol and aureomycin are much more effective against the rickettsioses than *p*-aminobenzoic acid, it might have been well to give this drug much less consideration than was devoted to it.

The book can be highly recommended.

**Landois-Rosemann Physiologie des Menschen mit besonderer Berücksichtigung der chemischen und pathologischen Physiologie.** Twenty-sixth edition. By Dr. Med. Hans-Ulrich Rosemann. Price, 46.60 German marks. Pp. 958, with 241 illustrations. Urban & Schwarzenberg, Thierschstrasse 11, München 2; Meinekestr. 13, Berlin W.15, 1950.

The first edition of this standard textbook on physiology was published in 1879 by Leonard Landois. This twenty-sixth edition follows the classical course in discussing the question of general physiology, but all in all it lacks the detail that one has come to expect in a modern textbook of physiology. As an example, the reviewer was unable to find any reference to the newer concepts dealing with the understanding of cardiac arrhythmias, as auricular flutter or fibrillation.

The book is well illustrated by many diagrams. The references are mostly of recent origin and are by no means limited to the German literature. The subject is covered in a fashion which would certainly leave the medical student with a fairly good knowledge of general physiology, but the volume is not adequate as a source of reference for the practicing physician.

**The Bases of Human Behavior: A Biologic Approach to Psychiatry.** By Leon J. Saul, M.D. Price, $4. Pp. 150, with illustrations. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5, 1951.

This book presents an introduction to the basic theories of dynamic psychiatry and considers the relation of these theories to present-day concepts of physiology, with particular reference to new theories concerning the physiologic effects of stress. Stress is considered to be either of a reactive type arising from the conflict of the person with his environment or of an inner type arising from the basic emotional conflicts of the person developed in childhood.

An attempt is made to follow the impact of psychic and emotional tension through to physiologic end-point reactions on body tissues.

The style of the book is simple and clear. It should be of considerable value to beginning students of medicine in clarifying the theoretic concepts which underlie the expanding field of psychosomatic medicine.

**Conference on Infancy and Childhood: Problems of Infancy and Childhood: Transactions of the Fourth Conference, March 6-7, 1950, New York.** Edited by Milton J. E. Senn, M.D. Price, $2.50. Pp. 181. Josiah Macy, Jr., Foundation, 565 Park Ave., New York 21, 1951.

On March 6 and 7, 1950, apparently, a group of especially invited guests held a two-day meeting in New York under the auspices of the Josiah Macy, Jr., Foundation, for the purpose of discussing certain problems of infancy and childhood. In opening the conference, Dr. Frank Fremont-Smith, the Medical Director of the Foundation, announced that this particular group would hold annual two-day meetings for five years. Hence this book is the merest fragment of what may appear later.

Three subjects were considered: "Cultural Determination of Parental Attitudes," "Consideration of Problems in the Ontogeny of Family Life and Social Adjustments in Various Infrahuman Animals," and "Working Toward Healthy Personality." Each was introduced by a competent authority and then discussed by equally competent authorities who had been invited to attend the meeting. What each of these authorities actually said now appears in print.

The result is a curious hodgepodge; some of the discussions are interesting; others are amusing when the speakers successfully tried to be funny, and still others have little to do with the points involved. To this reviewer, the results would have been much more valuable had a skilful editor, from the material which here occupies 181 pages, prepared a book of less length which gave a clearer and more readable account of the conclusions drawn and the opinions expressed by this intelligent group of experts when they discussed, informally, around a table, three such interesting topics.

As one reads the book in its present form and attempts to imagine himself hearing and seeing its dialogue enacted before him—even staged with appropriate casting, scenery, and costumes—he is inclined to regard the final curtain speech as one of the best in the play and to applaud it, *con amore:* "Unless there is objection I should like to adjourn the Conference."

**The Kidney: Structure and Function in Health and Disease.** By Homer W. Smith, Sc.D,. M.S. Price, $12.50. Pp. 1049 with 157 illustrations. Oxford University Press, 114 5th Ave., New York 11, 1951.

Smith's new book on the kidney is welcomed. It is much more extensive in its coverage of the subject than was the previous book by the same author. In many respects this may be a short-coming. Although the discussions and reviews of experimental studies by others are extensive, the data are not presented critically in many instances. This is unfortunate, since readers are especially interested in the author's opinion of the validity and significance of much of the physiologic work of other investigators. This uncritical approach is frequently the result of an attempt to present a subject in great detail or to review the literature comprehensively on a subject, such as the kidney. Another shortcoming in the monograph is the inadequate presentation of clinical material on renal disease and renal function as found in general practice. It probably is difficult for one not managing patients to evaluate clinical papers critically and adequately, especially when it is realized that the bibliography at the end of the monograph contains 2,300 publications listed in 142 pages.

The physiologic discussions are good and are supported by many diagrams and illustrations. The renal-function tests and clearances are discussed in some detail, almost all the more important papers on this subject at least being mentioned. The author, in these as in his own papers, has correlated as much as possible the relations of structure of the nephron to nephronic function. Abnormal as well as normal states are presented. This book should be read and studied by all interested in renal function and disease. It is unfortunate that the author did not sacrifice completeness for detailed critical discussion of the more important functional problems of normal and abnormal renal states. The reader will, however, find almost every aspect of renal function presented to some extent in this book. The monograph should serve as a good reference book on kidney problems, written by an authority on renal physiology.

**Allergy: Facts and Fancies.** Preface by Dr. Austin Smith. By Samuel M. Feinberg, M.D. Price, $2.50. Pp. 173. Harper & Brothers, 49 E. 33d St., New York 16, 1951.

Dr. Feinberg's little book is good; it has only 173 short pages, and it is printed in good taste. The story is told in simple language; it is factual; it is complete, and, best of all, it is free of those sensational aspects of allergy which so often distort the general picture.

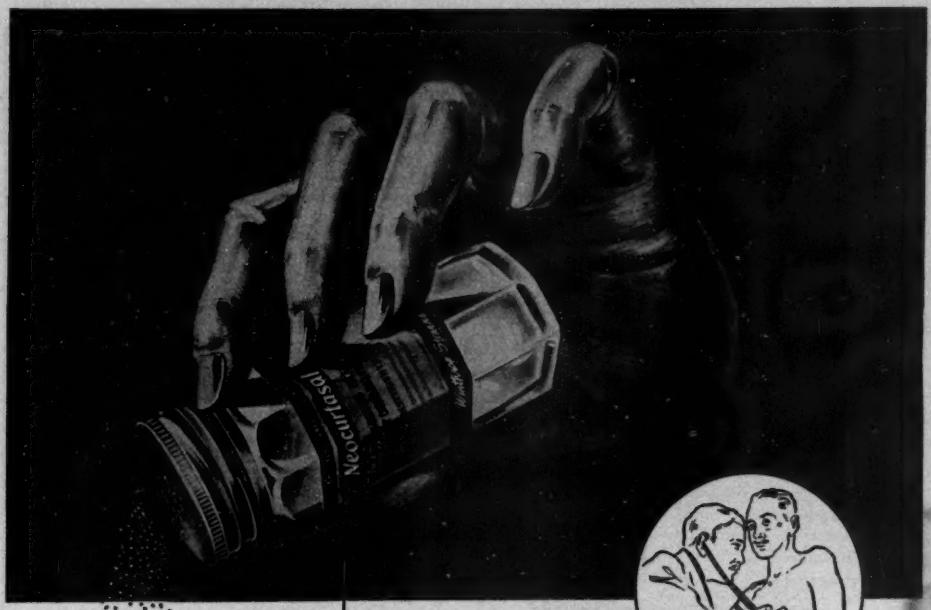
Dr. Feinberg has handled the various problems skilfully. The nature and the actions of drugs are clear, but the book is not a manual for self-medication. "Not everyone who wheezes has asthma." This statement is controversial, for others declare that asthma means a wheeze and a wheeze means asthma, but that "all is not allergy that wheezes."

The gadgets that are advised for filtering air, and many other fancies, are discussed with common sense. Some of them are useful.

Kapok, called "silk floss," deserves a little more emphasis, for it is of great practical importance in asthma. It is the growth of molds (fungi) upon the kapok fibers that breaks them down to a fine dust. Old, moldy kapok can be dangerous to those who have the "allergic constitution."

The term "intrinsic allergy" is confusing, for "allergy" means a sensitiveness to dusts or possibly foods outside the patient ("extrinsic"), whereas the term "intrinsic" means that the cause of trouble is something inside the patient which he carries with him at all times and to all places. The nature of "intrinsic" asthma represents the real problem.

As a whole, this little book will be helpful to every physician who must take care of patients with hay fever, asthma, or eczema, and it will be helpful to the patients also. It is recommended highly.



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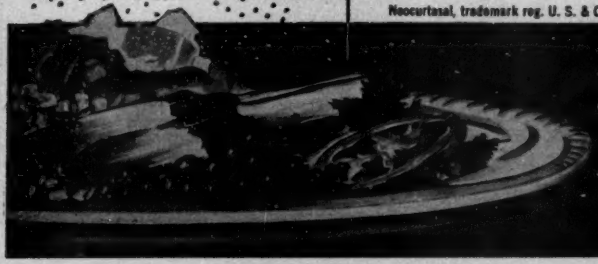

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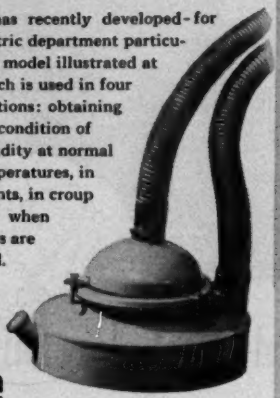

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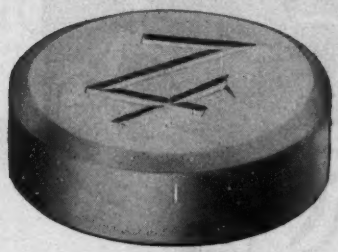

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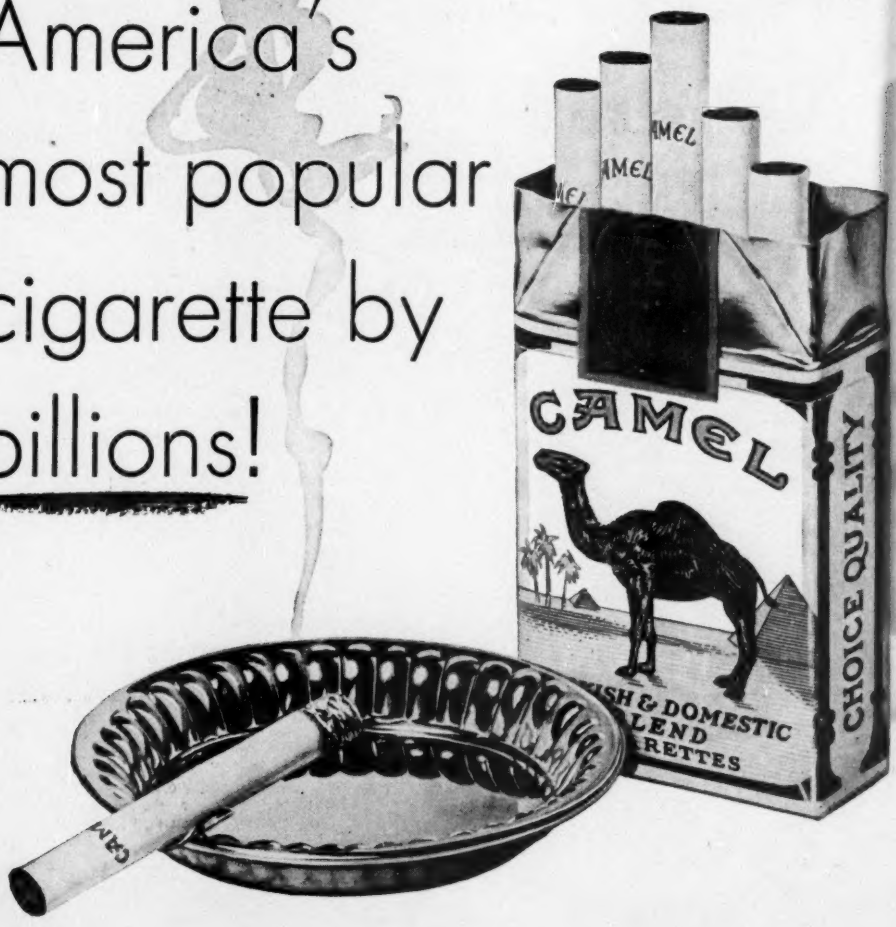

*Discussion: Place of blood, plasma and plasma "extenders" in treatment of severely burned patients.*

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